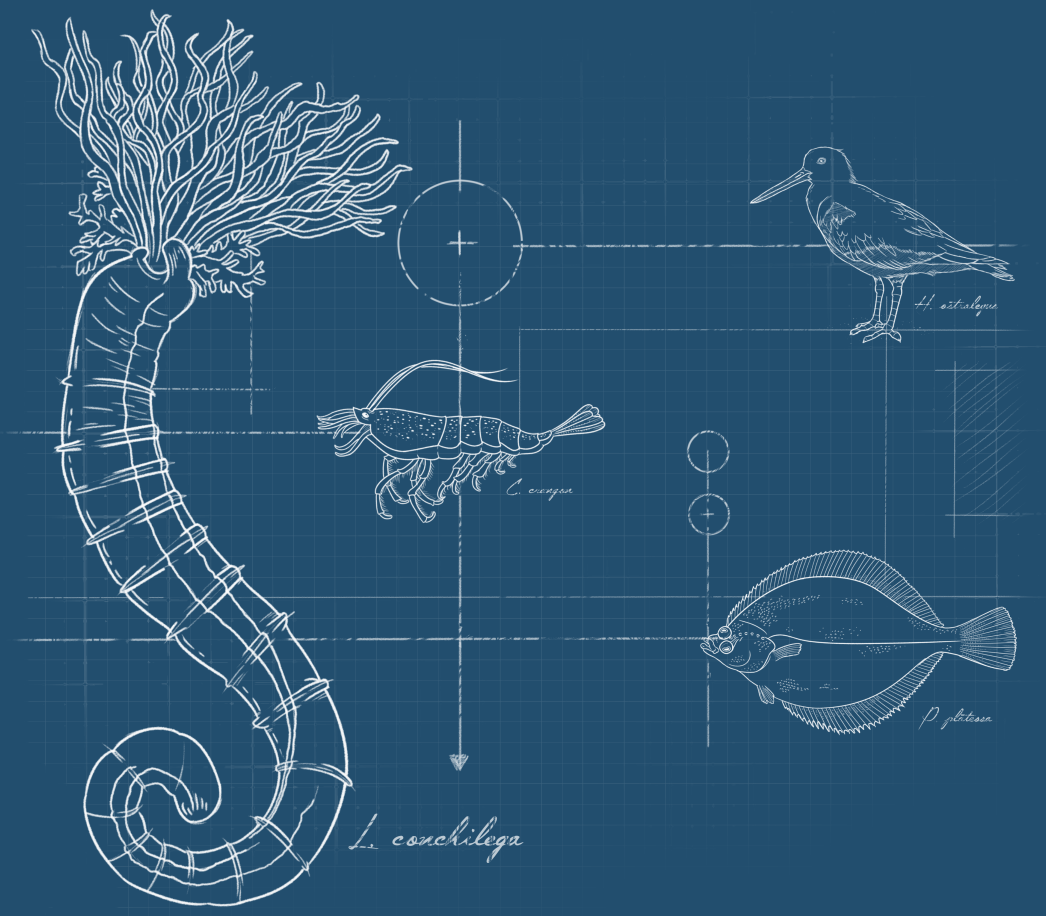
The importance of *Lanice conchilega* reefs in trophic linkages in intertidal areas



Bart De Smet

THE IMPORTANCE OF *LANICE CONCHILEGA* REEFS IN TROPHIC LINKAGES IN INTERTIDAL AREAS

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THE IMPORTANCE OF *LANICE CONCHILEGA* REEFS IN TROPHIC LINKAGES IN INTERTIDAL AREAS

Het belang van Lanice conchilega *riffen in voedselwebinteracties in intergetijdengebieden*

Bart De Smet

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Academic year 2015 - 2016

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And the arms of the ocean are carrying me
And all this devotion was rushing out of me
And the crashes are heaven for a scientist like me
But the arms of the ocean delivered me

- Florence Welch -

Dankwoord

213 weken, 1491 aardomwentelingen, of 35784 uur, dat is de tijd die ik nodig had om te bevallen van dit werk. Tussen het schrijven van de eerste zin van mijn doctoraatsvoorstel en de laatste punt van de finale thesis lag er een mooie periode; een periode waar ik nu al met heel veel plezier op terugblik. De afgelopen vier jaar hebben een sterke positieve invloed op me gehad, en dit zowel op mij als wetenschapper en als persoon. Zoals dat echter gaat met bevallingen, verliep deze periode ook met vallen en opstaan. Doctoreren is namelijk geen '*nine-to-five*' job. Het is iets wat onder je huid kruipt. Iets wat je bezighoudt, je gijzelt en op de duur een deel wordt van jezelf. Na die vier jaar is "*Lanice conchilega*" voor mij geworden wat $E=mc^2$ is voor Albert Einstein of wat Harry Potter is voor J.K. Rowling: allen onlosmakelijk verbonden, een alter ego als het ware. Doctoreren is meer dan wat stalen verzamelen, ze uitwerken en er een hele resem testjes op loslaten om dan toch maar dat ene wat je zo graag wil aantonen te kunnen aantonen. Doctoreren is een vorm van zelfontwikkeling. Het is jezelf leren kennen, je grenzen aftasten en ze af en toe overschrijden. Het is jezelf tegenkomen en je zelf verrassen. Het is het slechtste in jezelf bovenhalen om het beste te bereiken (of was het nu andersom). Het is jezelf verrijken, opwaarderen en verstevigen. Het is jezelf iPhonegewijs doen evolueren naar een betere versie. Er wordt ook zo vaak gezegd dat doctoreren wegkwijnen is achter een computerscherm, dat doctoreren het leiden is van een eenzaam bestaan. En dat is het ook! Soms. Héél af en toe. Het is maar wat je er zelf van maakt. Ik heb nooit het gevoel gehad dat ik er alleen voor stond - in tegendeel - en daar heb ik dan ook veel kracht uit geput. Ik kan me haast geen betere omkadering inbeelden. Van mijn begeleiders en collega's tot mijn naaste vrienden en familie, stuk voor stuk speelden ze een rol in het schrijven van dit werk. En dit wil ik dan ook niet onopgemerkt laten voorbij gaan.

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'Ge maakt er een brij van, maar de natuur is ook een brij'

- M. Vincx -

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'Weg met dat rif!'

- J. Vanaverbeke -

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'Stress tijdens mijn zwangerschap! Straks is het een homo!'

- U. Braeckman -

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'Sometimes a scream is better than a thesis'

- A. Van Kenhove -

De afgelopen vier jaar was meer dan louter werken. Dit doctoraat gaf me ook de mogelijkheid om onder andere de schoonheid van Zweedse fjorden te ontdekken, te

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'Work hard, play hard'

- B. De Smet -

woordenboek. En dat is niet enkel van toepassing op dit doctoraat; hoe zou de wereld eruit zien zonder uitdagingen en wat is een mensenleven zonder een *Bucket List*…

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Summary

Living organisms can have important impacts on physical and chemical processes occurring in their environment, as already stated by Charles Darwin in 1881 after having observed burrowing earthworms. However, the role of species in modifying habitats was only systematically defined in the late 20th century when Jones and co-authors introduced the concept of "*ecosystem engineers*": organisms that directly or indirectly alter the availability of resources to other species, by modifying the physical environment. In the marine environment, ecosystem engineers show a high variety and have representatives in all kinds of habitats, ranging from coastal areas (*e.g.* burrowing mangrove crabs) up to the open waters of oceans and extreme environments such as the deep sea (*e.g.* zooplankton producing faecal pellets). Since ecosystem engineers tend to prevail in stressful environments (characterised by strong abiotic forces such as wave action or drought), coastal sediments harbour particularly large amounts of ecosystem engineering species such as sea grasses, burrowing invertebrates or feeding sting rays.

Some ecosystem engineers are capable of constructing biogenic reefs: solid structures created by an accumulation of organisms and forming a community or habitat which is very different from its surroundings. A prime example is the reefs constructed by the tube-building polychaete *Lanice conchilega* (Terebellidae), a well-known and widely distributed ecosystem engineer in soft-bottom environments. These *L. conchilega* reefs have profound structuring impacts on the benthic environment by altering the biogeochemical and physical properties of the sediment, which consequently affects the community composition, abundance and species richness of its environment.

Several studies have focussed on ecosystem engineers, biogenic reefs and on the polychaete *L. conchilega*, and they mainly contributed to our understanding of the role of these organisms in structuring their environment and the subsequent changes in the

interactions between biotic and abiotic components (*e.g.* bioturbation). Nevertheless, significant knowledge gaps remain, especially dealing with the link between ecosystem engineers and food webs. Therefore, this PhD thesis aims at improving our understanding of the structural and functional role of reefs constructed by the ecosystem engineer *L. conchilega* in temperate soft-substrate coastal ecosystems. The study focusses on the French side of the English Channel, where substantial reefs of *L. conchilega* occur. An overview of the remaining knowledge gaps is outlined in **Chapter 1**, along with an elaborate description of the focal organism *L. conchilega* and a description of the two study sites in this PhD: the Bay of the Mont Saint-Michel (BMSM) and Boulogne-sur-Mer (Boulogne).

Biogenic reefs composed of *L. conchilega* are important from a conservation point of view, because they noticeably increase the biodiversity in otherwise species-poor environments. Most research however focusses on the effect of *L. conchilega* on a single ecosystem component at a time and this link is investigated at the local scale. In order to understand the general ecological function of the reefs beyond the local scale, the integrated effect of intertidal *L. conchilega* reefs on the major bentho-pelagic components of a soft-bottom intertidal ecosystem was studied in **Chapter 2**. The different components of the benthos community (*i.e.* macro-, hyper-, and epibenthos) were compared between an area dominated by *L. conchilega* and an area without any bioengineering species. Additionally, the effect of different local environmental characteristics on the structuring role of the reef habitat was investigated by including the two study sites. All three investigated benthic assemblages were positively affected by the presence of the *L. conchilega* reef structures. The magnitude of the effect depended on the link between the benthic assemblage and the sediment and was largest for the macrobenthos and least strong for the hyperbenthos (small animals living in the water column close to the seabed). The macro- and epibenthic community composition (benthic organisms living in and on the surface of the sediment respectively) was mainly structured by tubeworm density and macrobenthic food availability respectively, while the hyperbenthic community was rather structured by biotic environmental variables (such as total organic matter, chlorophyll a and suspended particulate matter). For the macro- and epibenthos, the reef effect was more

pronounced in Boulogne than for in BMSM and was shown to be mainly driven by the higher tubeworm density in the intertidal area of Boulogne.

The high abundance, diversity and biomass of the macrofauna associated with *L. conchilega* render the reef areas exceptionally important feeding grounds for waders in otherwise uniform habitats, as shown in **Chapter 3**. Because of the high food availability, waders were attracted to the reef areas of the Bay of the Mont Saint-Michel and their densities in the reef exceeded densities in bare sand areas about 47 times. Based on faecal analysis, *L. conchilega* was the only identifiable polychaete species present in all bird species, however waders in the reef area tended to feed on the associated fauna, and especially crustaceans, rather than specifically on the tubeworm itself. The findings of this and the previous chapter reinforce the central role that *L. conchilega* reefs play in linking benthic, pelagic and air-borne compartments in soft-bottom intertidal areas.

While the importance of *L. conchilega* reefs for higher trophic levels was clearly demonstrated, there is no knowledge on the possible existence of reverse interactions; *i.e.* whether and to which extent predators affect the activity of *L. conchilega* within the reef habitat. A mesocosm experiment was set up to investigate the activity of the tubeworm in the absence and restricted and unrestricted presence of the predatory shrimp *Crangon crangon* (**Chapter 4**). The bioirrigation activity was quantified by the decrease of an artificially introduced bromide (Br) tracer, while the feeding activity was measured from the incorporation of ^{13}C via stable isotope analysis. The bioirrigation activity of the *L. conchilega* reef equalled about 30 L m^{-2} d^{-1} and was shown to remain unaffected in the presence of the predatory shrimp *C. crangon*. The food uptake of the tubeworm was however about three times lower in the unrestricted presence of *C. crangon*, presumably due to the retraction of the worm's body and tentacles in its tube, induced by physical contact with the predator. Hence, *C. crangon* exerts a trophic pressure on the tubeworm which affects its feeding activity, but not its bioirrigation activity, enabling the tubeworm to maintain its functional role in soft-bottom intertidal areas, even under high predatory pressure.

Notwithstanding the investigated bottom-up and top-down effects within bio-engineered *L. conchilega* reefs, the significance of the common and often influential process of ecosystem engineering on the food web structure and dynamics remains largely unknown. Moreover, most studies dealing with this issue have a theoretical nature and empirical evidence is largely lacking. **Chapter 5** combined a classical and a more integrative stable isotope approach to explore the potential effect of *L. conchilega* reefs on the structure of the macroscopic soft-bottom intertidal food web of the two study locations. Despite the structural impacts of the ecosystem engineer on the associated macrofaunal community as reported in Chapter 2, the presence of *L. conchilega* reefs only has a minor effect on the structure of the food web. The isotopic niche width of the consumer communities of reef and control areas are highly similar, implying that consumer taxa do not shift their diet when feeding in a *L. conchilega* reef. Besides, species packing and hence trophic redundancy were not affected, pointing to an unaltered stability of the food web in the presence of *L. conchilega*. The ecosystem engineer's influence on the water column is probably too limited to substantially stir the global structure of the soft-bottom intertidal food web, which is mainly driven by water column-derived primary production.

Whereas the study in Chapter 5 looked at qualitative aspects, another important aspect of food web research consists in the quantification of food web flows, which yields a more thorough approximation of complex food webs. Therefore, **Chapter 6** makes use of linear inverse models (LIMs) to present a comprehensive analysis of carbon cycling and dynamics of the food web of a soft-bottom intertidal area in the presence and absence of *L. conchilega* reefs; focussing on the macrofaunal part of the food web. The food web models were based on an empirical dataset from two study sites and two time periods, consisting of biomass and stable isotope data, and general physiological constraints from the literature. The carbon input into reef food webs was about 40 times higher compared to bare sand areas and was mainly derived from organic matter in the water column. The tubeworm focussed the organic matter which is produced in an area at least 15 times larger than the reef itself. Although most of the organic matter input towards these reefs was consumed by suspension feeding macrofauna, particularly *L. conchilega* itself, the worm was not an important source of carbon for other macrofaunal

organisms. Rather, the tubeworm reinforces the bentho-pelagic coupling by establishing reef structures which act as a trap of organic matter, resulting in an overall high macrofaunal biomass in the presence of the tubeworm, and much more diverse and well-organised food webs.

The gained knowledge on the structural and functional role of the ecosystem engineer *L. conchilega* in natural soft-substrate coastal ecosystems is summarised and integrated in **Chapter 7**. This chapter is revolved around a conceptual diagram and discusses the potential of *L. conchilega* in linking community composition, feedback effects, food web structure and carbon cycling at the ecosystem level and beyond the local scale. The combined knowledge emphasises the added value of *L. conchilega* reefs in otherwise uniform intertidal areas. Additionally, the engineering effects of *L. conchilega* on the physical, biological and socio-economic aspects of the ecosystem are compared to the engineering effects of a variety of other ecosystem engineers. Whereas this PhD thesis provides one of the first attempts to link food web structure/stability and ecosystem engineering in soft-bottom intertidal areas, the discussion thoroughly elaborates on the incorporation of these two research areas.

In conclusion, the ecosystem engineer *Lanice conchilega* facilitates the bentho-pelagic coupling in soft-bottom intertidal areas and it was proven to strongly affect the faunal community composition, diversity, abundance and biomass, even under high predatory pressure. Notwithstanding this strong structural and functional role provided by the *L. conchilega* reefs, no direct effects on the overall structure or stability of the intertidal food web were detected. The magnitude of the links between different food web compartments however is modified by the increased carbon input in the presence of the reefs.

Samenvatting

Levende wezens kunnen een belangrijke impact hebben op de fysische en chemische processen in hun omgeving, zoals reeds aangegeven door Charles Darwin in 1881 na zijn observaties op gravende aardwormen. Nochtans werd de rol van soorten in het bewerken van hun habitat pas stelselmatig gedefinieerd vanaf het einde van de 20ste eeuw, nadat Jones en coauteurs het concept van "ecosysteemingenieurs" introduceerden. Dit zijn organismen die op een directe of indirect manier de beschikbaarheid van bronnen voor andere soorten kunnen beïnvloeden door hun fysische omgeving te veranderen. In het mariene milieu manifesteren ecosysteemingenieurs zich in een brede waaier aan vormen en hebben ze vertegenwoordigers in alle soorten habitats, gaande van kustzones (*bv.* gravende krabben in mangroves) tot de open wateren van de oceanen en extreme milieus (*bv.* de productie van fecale pellets door zoöplankton). Aangezien ecosysteemingenieurs het goed doen in stressvolle omstandigheden (gekenmerkt door sterke abiotische factoren zoals golfslag of droogte), herbergen kustgebieden bijzonder hoge aantallen ecosysteemingenieur soorten zoals onder andere zeegrassen, gravende ongewervelden en pijlstaartroggen.

Sommige ecosysteemingenieurs zijn in staat om biogene riffen te creëren: harde structuren die opgebouwd worden uit een accumulatie van organismen en die een gemeenschap of habitat vormen die sterk verschilt van de omgeving. Een uitstekend voorbeeld hiervan zijn de riffen opgebouwd door de schelpkokerworm *Lanice conchilega* (Polychaeta, Terebellidae), een goed bestudeerde en breed verspreide ecosysteemingenieur in zachte substraten. Deze *L. conchilega* riffen hebben een diepgaande structurerende impact op het benthische milieu doordat ze de biogeochemische en fysische eigenschappen van het sediment veranderen, wat op zijn

beurt zowel de gemeenschapssamenstelling, de abundantie als de rijkdom aan soorten in de omgeving beïnvloedt.

Talrijke studies hebben zich gefocust op ecosysteemingenieurs, biogene riffen en op de polychaete *L. conchilega*. Deze studies droegen vooral bij aan onze kennis omtrent de rol van deze organismen in het structureren van hun omgeving en de daaruit volgende veranderingen in de interacties tussen biotische en abiotische componenten. Er bestaan echter nog heel wat leemtes in onze kennis over deze organismen en in het bijzonder omtrent de link tussen ecosysteemingenieurs en voedselwebben. Deze thesis heeft dan ook als doel meer inzicht te verwerven in de structurele en functionele rol van riffen opgebouwd door deze ecosysteemingenieur in gematigde kustecosystemen. De studie focust op de Franse zijde van het Kanaal omwille van de prominente aanwezigheid van aanzienlijke *L. conchilega* riffen. **Hoofdstuk 1** geeft een overzicht van de resterende leemtes in onze kennis en beschrijft uitgebreid het modelorganisme *L. conchilega* en de twee studiegebieden in dit doctoraat: de Baai van de Mont Saint-Michel (BMSM) en Boulogne-sur-Mer (Boulogne).

Biogene riffen opgebouwd door de kokerworm *L. conchilega* zijn belangrijk vanuit een natuurbehoudsstandpunt aangezien ze zorgen voor een opmerkelijke toename in de biodiversiteit van soortenarme milieus. De meerderheid van het onderzoek focust zich echter op het effect van *L. conchilega* op een enkele ecosysteem component per keer en dit effect wordt meestal onderzocht op een lokale geografische schaal. Teneinde de algemene ecologische functie van een rif te achterhalen op grotere schaal, werd in **Hoofdstuk 2** het effect van intertidale *L. conchilega* riffen op de belangrijkste benthopelagiale componenten van een kustecosysteem bestudeerd. De verschillende componenten van de benthosgemeenschap (*i.e.* macro-, hyper- en epibenthos) werden vergeleken tussen een gebied dat gedomineerd werd door *L. conchilega* en een gebied zonder bio-ingenieurs. Daarnaast werd het effect van verschillende lokale omgevingskarakteristieken onderzocht door inclusie van twee studiegebieden. De drie bestudeerde benthische componenten werden positief beïnvloed door de aanwezigheid van de *L. conchilega* structuren. De grootte van het effect was afhankelijk van de link tussen de benthische gemeenschap en het sediment en was het grootst

voor het macrobenthos en het minst uitgesproken voor het hyperbenthos (kleine organismen zwemmend in het onderste deel van de waterkolom). De samenstelling van de macro- en epibenthos gemeenschap (organismen die zich respectievelijk in of op het bodemoppervlak bevinden) werd hoofdzakelijk gestructureerd door respectievelijk kokerwormdensiteit en macrobenthos beschikbaar als voedsel, terwijl de hyperbenthos gemeenschap eerder gestructureerd werd door biotische omgevingsvariabelen (zoals totale organische stof, chlorofyl a en gesuspendeerde deeltjes). In het geval van het macro- en epibenthos was het rif effect sterker uitgesproken in Boulogne in vergelijking met de BMSM, wat hoofdzakelijk een gevolg is van de hogere densiteit van de *L. conchilega* riffen in het intergetijdengebied van Boulogne.

De hoge aantallen, diversiteit en biomassa van de macrofauna geassocieerd met *L. conchilega*, maken de rifgebieden uitzonderlijk belangrijke voedselgronden voor wadvogels in de anders uniforme zandwoestijn van de Baai van de Mont Saint-Michel, zoals werd aangetoond in **Hoofdstuk 3**. Als gevolg van de hoge voedselbeschikbaarheid werden wadvogels aangetrokken tot het rifgebied en hun aantallen in het rif oversteeg deze in louter zandgebieden zo'n 47 keer. Ondanks dat fecale analyse aantoonde dat *L. conchilega* de enige identificeerbare polychaet was in het dieet van alle vogels, verkozen wadvogels zich eerder te voeden met de geassocieerde macrofauna (en in het bijzonder met schaaldieren) dan specifiek met de kokerworm zelf. De resultaten van dit en het vorige hoofdstuk versterken de kennis omtrent de centrale rol die *L. conchilega* speelt in het koppelen van de bodem-, waterkolom- en luchtgebonden compartimenten in het intergetijdengebied van kustzones.

Terwijl het belang van *L. conchilega* riffen voor hogere trofische niveaus duidelijk werd bewezen, hebben we nauwelijks kennis van de potentiële omgekeerde interacties; *i.e.* of, en in welke mate, predatoren de activiteit van *L. conchilega* in een rifhabitat beïnvloeden. Daarom werd een mesocosmos experiment opgezet om de activiteit van *L. conchilega* in de afwezigheid en de directe en indirecte aanwezigheid van de Noordzeegarnaal *Crangon crangon* te onderzoeken (**Hoofdstuk 4**). De bioirrigatie activiteit werd gekwantificeerd door de afname van een artificieel geïntroduceerde

bromide (Br^-) tracer in de waterkolom te meten, terwijl de voedingsactiviteit bepaald werd op basis van de opname van ^{13}C via een stabiele isotopen analyse. De bioirrigatie activiteit van een *L. conchilega* rif bedroeg ongeveer 30 L m^{-2} d^{-1} en werd niet beïnvloed door de aanwezigheid van de predator *C. crangon*. De voedselopname van de kokerworm daarentegen was ongeveer drie keer lager in de directe aanwezigheid van *C. crangon*, wat vermoedelijk het gevolg was van de terugtrekking van de worm en zijn tentakels in de koker als reactie op een fysiek contact met de predator. *C. crangon* oefent dus een trofische druk uit die een invloed heeft op de voedingsactiviteit maar niet op de bioirrigatie activiteit van de kokerworm, hetgeen *L. conchilega* in staat stelt om zelfs onder hoge predatiedruk zijn functionele rol in het intergetijdengebied van kustzones te handhaven.

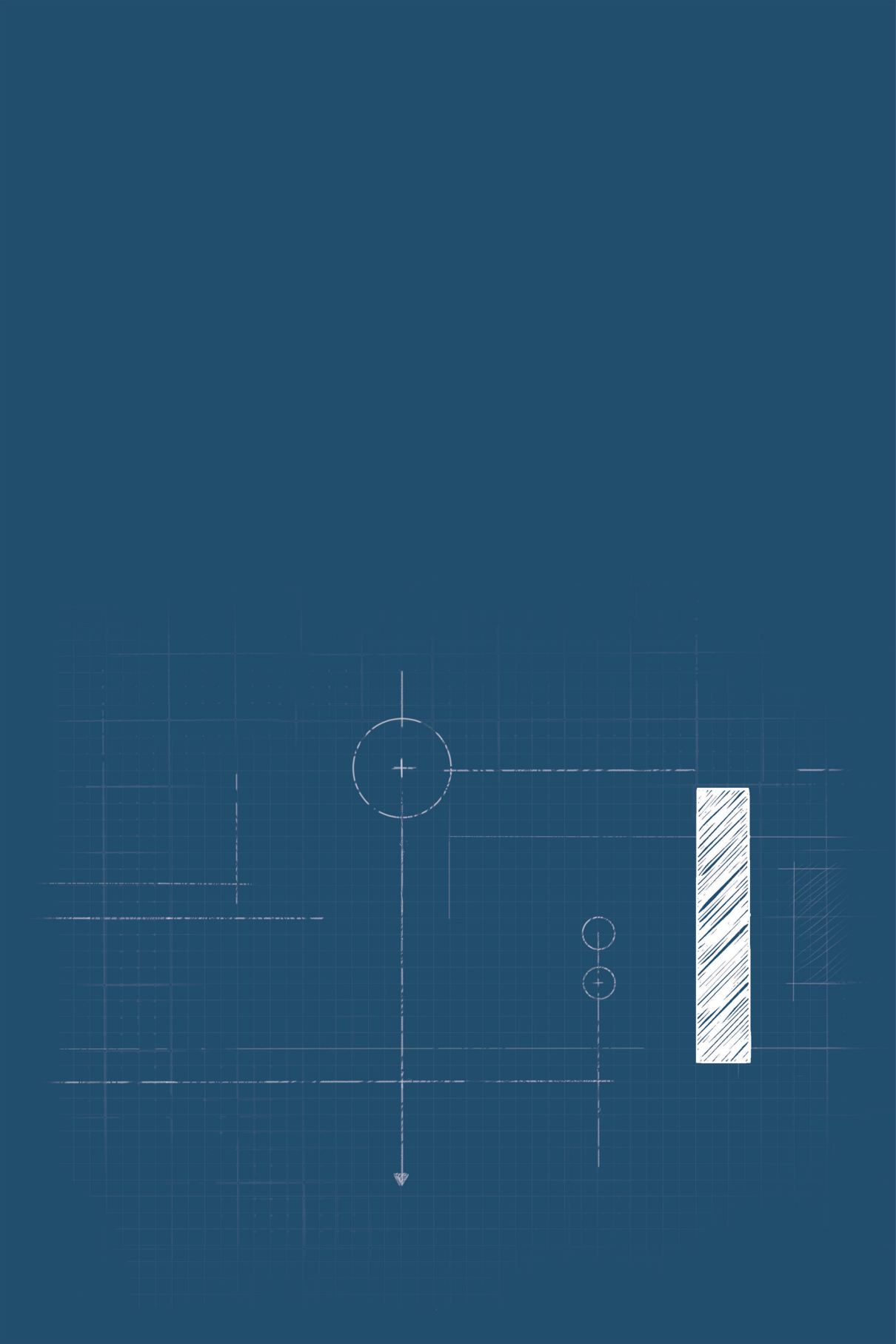
Ondanks de onderzochte bottom-up en top-down effecten in biogene *L. conchilega* riffen, blijft het belang van het alomtegenwoordige en vaak invloedrijke proces van ecosysteem engineering op de structuur en de dynamiek van een voedselweb grotendeels onbekend. Daarnaast zijn de meeste studies aangaande de link tussen ecosysteem engineering en voedselwebben theoretisch van aard en ontbreekt empirisch bewijs. **Hoofdstuk 5** combineert een klassieke en een meer geïntegreerde stabiele isotoop benadering om het potentiële effect van *L. conchilega* riffen op de structuur van het intertidale, macroscopische voedselweb te onderzoeken voor de twee studiegebieden. Ondanks de structurele impact van de ecosysteemingenieur op de gemeenschap van geassocieerde macrofauna, zoals aangetoond in Hoofdstuk 2, heeft de aanwezigheid van *L. conchilega* riffen een zeer gering effect op de voedselwebstructuur. De breedte van de isotopen niche van de consumentengemeenschap in de rif- en controlegebieden is quasi gelijk, wat impliceert dat de consumenten hun dieet niet wijzigen wanneer ze zich voeden in een *L. conchilega* rif. Tevens werd de opeenpakking van soorten en bijgevolg het aantal soorten met een gelijkaardige trofische ecologie niet beïnvloed, wijzend op een onveranderde voedselwebstabiliteit in de aanwezigheid van *L. conchilega*. De invloed van de ecosysteemingenieur op de waterkolom is waarschijnlijk te beperkt om de globale structuur van het intertidale voedselweb, dat hoofdzakelijk gestuurd wordt door primaire productie uit de waterkolom, te wijzigen.

Terwijl de studie in Hoofdstuk 5 zich focust op de kwalitatieve aspecten, is een ander belangrijk aspect van voedselwebonderzoek de kwantificatie van de koolstofstromen in een voedselweb, wat een grondigere en meer complete benadering oplevert van complexe voedselwebben. Daarom wordt in **Hoofdstuk 6** gebruik gemaakt van lineaire inverse modellen (LIMs) om de koolstofdoorstroom en -dynamiek van een intertidaal voedselweb (met een focus op macrofauna) in de aan- en afwezigheid van *L. conchilega* uitgebreid te analyseren. De voedselwebmodellen zijn gebaseerd op een empirische dataset, bestaande uit biomassa en stabiele isotopen data van de twee studiegebieden en twee seizoenen, en algemene fysiologische restricties uit de literatuur. De koolstofinput in een voedselweb in de aanwezigheid van een rif was ongeveer 40 keer hoger dan in de afwezigheid van een rif en was hoofdzakelijk afkomstig van organisch materiaal uit de waterkolom. De kokerworm concentreerde het organisch materiaal dat geproduceerd werd in een gebied dat op zijn minst vijftien keer groter was dan het rif zelf. Alhoewel het merendeel van de input aan organisch materiaal in een rif geconsumeerd werd door suspensievoeders, en in het bijzonder *L. conchilega* zelf, vormde de worm geen belangrijke koolstofbron voor andere organismen. *Lanice conchilega* versterkt dus eerder de bentho-pelagische koppeling door het creëren van structuren die fungeren als een val voor organisch materiaal, wat resulteert in een algehele hogere biomassa van macrofauna en veel diversere en beter georganiseerde voedselwebben.

De verworven kennis over de structurele en functionele rol van de ecosysteemingenieur *L. conchilega* in de natuurlijke zachte substraten van kustecosystemen wordt samengebracht in **Hoofdstuk 7**. Dit hoofdstuk is opgebouwd rond een conceptueel diagram en bediscussieert het potentieel van *L. conchilega* in het koppelen van de gemeenschapssamenstelling, feedback effecten, voedselwebstructuur en koolstofstromen op het niveau van het ecosysteem en op grotere geografische schaal. De gecombineerde kennis benadrukt de toegevoegde waarde van *L. conchilega* riffen in anders homogene intergetijdengebieden. Bovendien worden de ingenieurseffecten van *L. conchilega* op de fysische, biologische en socio-economische aspecten van het ecosysteem vergeleken met de ingenieurseffecten van verscheiden andere ecosysteem ingenieurs. Vermits deze doctoraatsthesis een van de eerste pogingen is om

voedselwebstructuur/stabiliteit te linken aan ecosysteem engineering in kustecosystemen, gaat de algemene discussie dieper in op de integratie van deze twee onderzoeksdomeinen.

We kunnen besluiten dat de ecosysteemingenieur *Lanice conchilega* de benthopelagische koppeling in intergetijdengebieden bevordert en een sterke invloed heeft op de gemeenschapssamenstelling, diversiteit, abundantie en biomassa van de geassocieerde fauna; zelfs onder een hoge predatiedruk. Ondanks de sterke structurele en functionele rol van de *L. conchilega* riffen, hebben deze structuren geen directe impact op de algemene structuur of stabiliteit van het intertidale voedselweb. De grootte van de koppelingen tussen verschillende voedselwebcompartimenten wijzigt echter door de verhoogde koolstofinput in de aanwezigheid van de riffen.



Chapter 1

General Introduction

Ecosystem engineers

Living organisms can have important impacts on physical and chemical processes occurring in their environment (Wright and Jones 2006). To stress the importance of environmental modification by organisms, two decades ago, Jones *et al.* (1994) introduced the concept of *ecosystem engineering* performed by so-called *ecosystem engineers*. They defined ecosystem engineers as *organisms that directly or indirectly modulate the availability of resources (other than themselves) to other species, by causing physical state changes in biotic or abiotic materials.* Next to this process, the direct transfer of resources by an organism to other species in the form of living or dead tissues is not considered ecosystem engineering, but is defined as trophic interactions. Thus, a critical characteristic of ecosystem engineers is that they change the quality, quantity and distribution of resources utilized by other species, excluding the engineer itself as a resource (Jones *et al.* 1994). A distinction is made between two types of engineers. Autogenic engineers (*e.g.* corals or trees in a forest) change the environment via their own physical structures; *i.e.* their living and dead tissues. Allogenic engineers (*e.g.* woodpeckers or beavers) change the environment by transforming living or non-living materials from one physical state to another (Jones *et al.* 1994).

In the marine environment, ecosystem engineers show a high variety and have representatives in all possible habitats ranging from coastal areas (*e.g.* burrowing mangrove crabs, Kristensen 2008) up to the open waters of oceans and extreme environments such as the deep sea (*e.g.* zooplankton producing faecal pellets in the Southern Ocean, Manno *et al.* 2015). As ecosystem engineers tend to prevail in stressful environments (characterised by strong abiotic forces such as wave action or drought, Jones *et al.* 1997), many coastal sediments harbour particularly large amounts of

ecosystem engineering species such as seagrasses and endobenthic invertebrates (Bouma *et al.* 2009a). Examples are as variable as sediment accretion and modification by intertidal sea grass beds (*e.g.* Bos *et al.* 2007) and the sediment reworking of feeding sting rays on intertidal sandflats (*e.g.* O'Shea *et al.* 2012).

By modifying, maintaining and/or creating habitats, ecosystem engineers greatly impact community composition, species richness and abundance of other species, both in positive and negative ways (Jones *et al.* 1997) (Fig. 1).

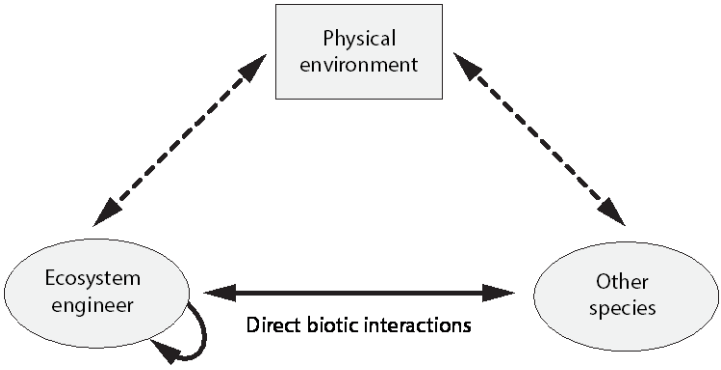


Figure 1. Diagrammatic representation of interactions of ecosystem engineers. The dashed lines represent the abiotic interactions. Adapted from Hastings et al. (2007)

Further, Gutiérrez and Jones (2006) showed that by affecting the physical characteristics within, on, or above a soil or sediment patch, ecosystem engineers can change the occurrence or rates of biogeochemical processes. For instance, polychaetes of the genus *Nereis* are capable of facilitating the flow of oxygen to the otherwise anoxic subsurface sediments by constructing burrows that increase the infiltration of overlying water into deeper sediment layers and by ventilating their burrows by means of bioirrigation via undulating body movements (Kristensen *et al.* 1991; Nielsen *et al.* 2004). The increased amount of oxygen in the sediment surrounding the burrow walls of *Nereis* enhances microbial nitrification (Nielsen *et al.* 2004) and subsequently facilitates the nitrate limited process of denitrification/nitrate reduction in the anoxic layers of the sediment patch (Kristensen *et al.* 1991; Nielsen *et al.* 2004). Changes in the biogeochemistry as a result of the activity of an ecosystem engineer are not necessarily confined to the local scale and

may have effects at the ecosystem level as illustrated by the introduction of the bioirrigating polychaete *Marenzelleria spp* in the Baltic Sea (Kauppi *et al.* 2015). Following an altered disturbance regime in the Baltic Sea (*i.e.* declining oxygen levels since the beginning of the 1980s; *e.g.* Conley *et al.* 2011) this opportunistic species showed an increased abundance. Nevertheless, the introduction of *Marenzelleria spp.* is hypothesized to counteract hypoxia, facilitating the return of native species (Karlson *et al.* 2011; Josefson *et al.* 2012) and hence showing positive effects with regards to the eutrophication of the Baltic Sea (Kauppi *et al.* 2015).

Ecosystem engineers and food webs

The study of trophic interactions (*i.e.* consumer-resource or predator-prey interactions) has dominated ecological research for as long as it exists. Nevertheless, in ecosystems, species interact with other species and with abiotic factors in many ways, of which trophic interactions are only one of many types (Hutchinson 1959; Berlow *et al.* 2004; Olff *et al.* 2009). Non-trophic interactions include, among others, habitat provisioning and ecosystem engineering and have been largely ignored by the food web community (Berlow *et al.* 2004). Research addressing both trophic and non-trophic interactions should however be encouraged since it provides the opportunity to explore more thoroughly the minimum detail required to explain the widest variety of observed food web patterns and dynamics (Berlow *et al.* 2004). Ecosystem engineering is one of the most common and influential kind of non-trophic interactions, and is nearly as universal as trophic interactions (Wright and Jones 2006). Moreover, it is believed to potentially alter the structure and dynamics of entire food webs (Olff *et al.* 2009). Sanders *et al.* (2014) present a conceptual framework identifying how ecosystem engineering can affect the links and nodes of food webs, and their overall organisation. The incorporation of non-trophic interactions in traditional food web studies is however only recently increasing (*e.g.* Olff *et al.* 2009; Kéfi *et al.* 2012), and up till now, the significance of ecosystem engineering on food web structure and dynamics remains largely unknown (Sanders *et al.* 2014). This lack of valuable knowledge can be attributed to an insufficient overlap of engineering and food web studies in the same systems (Sanders *et al.* 2014).

Besides the bottom-up impacts on the overall food web, the increased species diversity and abundances resulting from ecosystem engineering activities can be expected to give rise to top-down feedbacks from the impacted species to the engineer (Sanders *et al.* 2014). Jones *et al.* (1994) however argue that there may often be no direct feedback effects of the impacted species upon the engineer population, especially if the ecosystem engineers form no part of the flow of energy and material among trophically connected organisms (Jones *et al.* 1997). Nevertheless, some ecosystem engineers modify the physical environment, and at the same time belong to the food web to which the impacted species belong (*e.g.* Tribollet and Golubic 2011). This dual role of ecosystem engineers is potentially one of the most important bridges between trophic and non-trophic interactions (Sanders *et al.* 2014). When the ecosystem engineer is trophically coupled to the food web, a trophic feedback from the impacted species altering the ecosystem engineer's density and/or activity is expected (Sanders *et al.* 2014). A negative feedback is for instance reported for oyster reef habitats which create refugia for the oyster consuming mud crab *Panopeus herbstii* (Grabowski and Powers 2004).

Lanice conchilega as a study organism

The sandmason *Lanice conchilega* (Pallas, 1766), which is a well-known and widely distributed ecosystem engineer in soft-bottom environments (Rabaut *et al.* 2007a), will be the focal species in this thesis. *Lanice conchilega* was selected because it is an ideal model organism for studying the sediment-animal-interactions contributing to the strength of a benthic engineer in modifying its habitat and thereby affecting other species (Reise *et al.* 2009). This species is easily accessible and there was a good chance that it would be available for sampling during the entire study period. Moreover, *L. conchilega* is a good example of an ecosystem engineer structurally affecting its environment and at the same time forming part of the food web to which the impacted species belong.

Lanice conchilega is a tube-building worm belonging to the family of the Terebellidae (Annelida, Polychaeta) (Fig. 2). The worm's body has 150 to 300 segments (up to 250 – 300 mm long) and is divided in an inflated anterior region and a more slender posterior

region. Most segments have both noto- and neuropods and the latter bear uncini: small plates with a serrated outer edge by which the worm anchors itself to the wall of its tube. The truncated head is surrounded by various lobes and has many long, filiform, mobile tentacles (Hartmann-Schröder 1996). This tube-dwelling polychaete has a worldwide distribution and in Europe, where the largest populations can be found, it colonises a wide range of intertidal and subtidal sediments down to a depth of approximately 1900 m (Hartmann-Schröder 1996). The widespread distribution of *L. conchilega* may resemble a mixing of species (Marcano and Bhaud 1995), however their functional value is expected to be similar and mainly dependent on the structure and dynamics of their populations (Godet *et al.* 2008). *Lanice conchilega* is found in various sediments, but displays a preference for fine to medium-grained sediments (D50 = 100 to 500 μm) with a relatively high mud content (10 to 40%) (Degraer *et al.* 2006). The species creates a tube of sand grains and calcium carbonate particles (shell fragments) cemented into a strong, homogeneous mucus layer (Ziegelmeier 1952). A matured tube has a diameter of about 5 mm and a length of maximum 65 cm, but is usually no longer than 20 cm (Ziegelmeier 1952; Jones and Jago 1993). The top end of the tube protrudes some 1 to 4 cm above the sediment and is crowned with a fringe of filaments of mucus-bound coarse sand grains (Jones and Jago 1993). Upon physical disturbance, the worm can retreat very fast in its tube (Bergman and Hup 1992).

Figure 2. From left to right: the polychaete Lanice conchilega *without its tube (© M. Buschmann),* L. conchilega *tubes in an experimental setup, a* L. conchilega *reef patch in the Bay of the Mont Saint-Michel (France), and the* L. conchilega *reef of the intertidal area of Boulogne-sur-Mer (France)*

Lanice conchilega is a surface deposit feeder and has the ability to switch to suspension feeding in case the amount of food on the bottom is limited (Buhr 1976; Fauchald and Jumars 1979), which is especially advantageous to avoid competition in areas of high

densities (*i.e.* several thousand ind.m^{-2}; Buhr (1977)). As a suspension feeder, *L. conchilega* is less efficient in retaining small particles compared to suspension feeding bivalves such as oysters or mussels (Ropert and Goulletquer 2000). The tubeworm's filtration rate is positively linearly related to body size while the relative daily food intake decreases with growth (Buhr 1976). Moreover, the filtration rate of *L. conchilega* is optimal at a flow velocity of 15 cm.s^{-1} (Denis *et al.* 2007). So far, research has revealed that the polychaete's diet relies less on phytoplankton and more on organic matter sources linked to the sediment; *i.e.* microphytobenthos and macroalgal fragments (Lefebvre *et al.* 2009), and to some extent bacteria (Braeckman *et al.* 2012).

Biogenic *Lanice conchilega* reefs

Most soft-bottom intertidal areas contain limited amounts of three-dimensional abiotic structuring factors, but they can possess biogenic reefs. These are biotic surface structures which are mainly constructed by ecosystem engineers. According to Holt *et al.* (1998), biogenic reefs are "*Solid, massive structures which are created by accumulations of organisms, usually rising from the seabed, or at least clearly forming a substantial, discrete community or habitat which is very different from the surrounding seabed. The structure of the reef may be composed almost entirely of the reef building organism and its tubes or shells, or it may to some degree be composed of sediments, stones and shells bound together by the organisms.*" This biogenic reef definition was used by Holt *et al.* (1998), to select Special Areas of Conservation in the UK in the framework of the Habitats Directive[1] (as part of the Natura 2000 network[2]). Bivalve accumulations of *Mytilus edulis* and *Modiolus modiolus* and polychaete tube assemblages such as those of *Sabellaria* species and *Serpula vermicularis* are some of the most striking biogenic structures in coastal areas (Holt *et al.* 1998). *Lanice conchilega* can reach densities of several thousands of individuals per square meter and produces gentle mounds interspersed with shallow depressions (Ziegelmeier 1952; Carey 1987) (Fig. 2). Using the guidelines provided by Hendrik and Foster-Smith (2006), Rabaut *et al.* (2009) showed that all physical and biological characteristics are present to add *L. conchilega* to this list of reef building

[1] Directive 92/43/EEG of 21 May 1992 on the Conservation of Natural Habitats and of Wild Fauna and Flora, *PB L*206, 22 July 1992; http://ec.europa.eu/environment/nature/legislation/habitatsdirective/index_en.htm
[2] http://ec.europa.eu/environment/nature/natura2000/

species. Consequently, above a critical threshold of density and individual tube diameter, surfaces colonised by this species are defined as '*L. conchilega* reefs' (Rabaut *et al.* 2009). Nevertheless, it has to be kept in mind that the *L. conchilega* habitats have different developmental stages, ranging from '*L. conchilega* beds' with a low value for the physical, biological and temporal reef criteria in the scoring system of Rabaut *et al.* (2009) up to very pronounced biogenic structures that are very 'reefy'. The largest intertidal reefs are found in the Wadden Sea (Petersen and Exo 1999) and in France, especially in the bay of Veys (Ropert and Dauvin 2000) and the Bay of the Mont Saint-Michel (Godet *et al.* 2008).

Although *L. conchilega* builds elevated structures and changes the sedimentary environment, they can rapidly disappear (Holt *et al.* 1998; Callaway *et al.* 2010), especially in intertidal areas which are more prone to changing environmental characteristics and harsh winter conditions (Zühlke 2001). For instance Ziegelmeier (1964) and Strasser and Pieloth (2001) report the mass mortality of *L. conchilega* following exceptionally cold winters. Following population collapses, the tubeworm has however been shown to recolonize the intertidal flat by secondary dispersal of juveniles (Gunther 1999; Callaway *et al.* 2010). Important for the recruitment of pelagic tubeworm larvae is the prolonged existence of the sediment mounds created by *L. conchilega*, even after a period in which worms are absent from the mounds (up to > 100 days) (Rabaut *et al.* 2009; Borsje *et al.* 2014). The occasional mortality events however imply an ephemeral spatial pattern of *L. conchilega* populations in nature (Zühlke 2001; Rabaut *et al.* 2009). In this respect, *L. conchilega* reefs differ in comparison with other habitat structuring species such as mussels, which show relatively stable distribution patterns (Zühlke 2001). The ephemeral spatial pattern and the different developmental stages of *L. conchilega* aggregations illustrate that their reef status is still the subject of an on-going debate. Therefore, when it comes to policy making, there has been some reluctance to protect *L. conchilega* aggregations as reefs (Rabaut and Cliquet 2011). Nevertheless, it is widely accepted that all reef building organisms are, in some way or another, ephemeral by nature (Hendrick and Foster-Smith 2006) due to natural or anthropogenic disturbance.

The impact of *Lanice* on its environment

Lanice conchilega is a prime example of an organism proven to be both an autogenic and allogenic ecosystem engineer (Godet *et al.* 2008; Rabaut *et al.* 2009). The allogenic engineering of *L. conchilega* alters the biogeochemical properties of the environment mainly by its bio-irrigating activity (exchanging burrow water with the overlying water, *i.e.* 'piston-pumping'), hereby introducing O_2-rich water into the sediment (Forster and Graf 1995). The *L. conchilega*-induced oxygenation of deeper sediment layers affects the species composition of the smaller interstitial communities (Zühlke *et al.* 1998; Braeckman *et al.* 2011b) and has important repercussions on benthic respiration, nutrient release and denitrification (Braeckman *et al.* 2010). The autogenic engineering consists of the biogenic reef formation by *L. conchilega* (Carey 1987; Rabaut *et al.* 2009). High densities of the tubeworm change the sedimentary composition of its surroundings (Van Hoey 2006; Rabaut *et al.* 2007a) as a result of a change in the hydrodynamic regime: a so-called 'skimming flow' or deceleration of the water currents at the sediment-water interface (Luckenbach 1986). Further, the tubeworm aggregations increase the sediment consolidation and can elevate the seabed up to 80 cm above the surroundings by sediment trapping in between the protruding tubes (Degraer *et al.* 2008; Rabaut *et al.* 2009). The reef height is modelled to increase with the tube length of *L. conchilega* and with its population density (with a stagnating sediment accretion for population densities > 3000 ind.m^{-2}) (Borsje *et al.* 2014). The physical changes induced by *L. conchilega* result in the provision of new habitats and an attractive environment for a large array of species.

Several studies have examined the allo- and autogenic ecosystem engineering effect of *L. conchilega* on communities and they show biodiversity and/or abundance effects on different size and/or ecological groups, ranging from smaller meiofauna (*e.g.* Zühlke *et al.* 1998; Braeckman *et al.* 2011b) and associated macrobenthos (Zühlke *et al.* 1998; Van Hoey *et al.* 2008) up to (juvenile) (flat)fish (Rabaut *et al.* 2013) and birds (Carey 1987; Petersen and Exo 1999). Nematode abundances and survival are considerably higher in the close vicinity of individual *L. conchilega* tubes supposedly due to the improved sediment oxygen conditions (Zühlke *et al.* 1998; Braeckman *et al.* 2011b), the

construction of physical structures (*L. conchilega* tubes) that provide shelter from macrofaunal predation (Dauer *et al.* 1982), and the stimulated bacterial provisions in the mucous linings of the tube and/or in the sediment surrounding the tubes (Ziegelmeier 1952; Solan and Wigham 2005). Similarly, the macrobenthic community composition, species diversity and abundance was repeatedly shown to be altered in the presence of a *L. conchilega* reef, both for different intertidal study areas (a sand flat in the German part of the Wadden sea (Zühlke *et al.* 1998; Zühlke 2001), a sandy beach in South Wales, UK (Callaway 2006)) and subtidal study areas (the North Sea (Van Hoey *et al.* 2008), the Belgian Part of the North Sea (Rabaut *et al.* 2007a), and the Dutch North Sea (Coolen *et al.* 2015)). The increase in the number and density of species was shown to be positively related to the density of *L. conchilega* (Van Hoey *et al.* 2008). However the increase in the species richness stabilised or even decreased after reaching a critical density of *L. conchilega* (> 1500 ind. m^{-2}) (Rabaut *et al.* 2007a; Van Hoey *et al.* 2008). Besides, Rabaut *et al.* (2007a) visualised the general ecosystem engineering effect of *L. conchilega* for the benthic community in a so-called 'Babushka-like pattern': the highest variability in samples without *L. conchilega* and the lowest variability in samples with the highest tubeworm densities. This pattern indicates that *L. conchilega* restructures the species assemblage by expanding the realised niche of several species, rather than forming its own association (Rabaut *et al.* 2007a; Van Hoey *et al.* 2008), and implies that species which are responsible for the increase in the diversity belong to the overall species-pool of the habitat. The generally positive impact on the macrobenthos is due to several environmental changes: an increased oxygen supply in the surrounding sediments (Forster and Graf 1995), the function of the reefs as a refuge for epifaunal predation (Woodin 1978), the increased availability of attachment surfaces for larval and post-larval benthic organisms (Qian *et al.* 1999), and the modification of the hydrodynamic regime (Eckman *et al.* 1981).

Lanice conchilega not only affects infaunal communities; they are also attractive and important feeding grounds for flatfish and birds (*e.g.* Petersen and Exo 1999; Rabaut *et al.* 2013), and they are expected to be important in the diet of other epifaunal species such the brown shrimp *Crangon crangon*. Biotopes composed of tubes of the polychaetes *Chaetopterus* and *L. conchilega* were suggested to be important for several

commercial fish species (Rees *et al.* 2005) and *L. conchilega* constitutes an important prey item for juvenile plaice *Pleuronectes platessa* and sole *Solea solea* (Amara *et al.* 2001). On a small scale, the distribution of post-larval flatfishes is positively influenced by the tube building polychaete, both in intertidal (Rabaut *et al.* 2010) and subtidal (Rabaut *et al.* 2013) areas. Moreover, *S. solea* selects low tube density habitats over very high density reefs, most probably due to its more favourable hydrodynamic conditions (Rabaut *et al.* 2013). The attractiveness of the *L. conchilega* habitat lies in the increased food availability and the provision of shelter for flatfish. The findings of Rabaut *et al.* (2010; 2013) highlight the importance of *L. conchilega* habitats within flatfish nursery areas.

Finally, *L. conchilega* reefs are important feeding grounds for birds. Carey (1987) reported browsing traces of eider ducks feeding in shallow water on *Mytilus edulis* spat attached to *L. conchilega* tubes. Several other authors provided evidence for the importance of *L. conchilega* itself in the diet of birds (*e.g.* Goss-Custard and Jones 1976; Yates *et al.* 1993). Regarding the importance of the reef structures, birds in the Chausey archipelago (France) were shown to select *L. conchilega* reefs as a major feeding ground over other available areas (Godet *et al.* 2008). For instance, the attractiveness of the reefs for the oystercatcher *Haematopus ostralegus* is mainly due to the high abundances of macrobenthic invertebrates (especially the cockle *Cerastoderma edule*). An alteration of this reef habitat via clam cultivation was shown to decrease the abundance, species richness and diversity of the associated macrofauna, inducing a loss of attractiveness for feeding birds (Godet *et al.* 2009). In the German Wadden Sea, tidal flats dominated by *L. conchilega* were shown to be of overall importance as foraging areas for migratory birds (Petersen and Exo 1999). While for most waders in this area, the associated macrobenthos was of overriding importance in their diet, *L. conchilega* itself constituted the main food item for only a few species, especially gulls (Petersen and Exo 1999).

Soft-bottom intertidal areas

Although *L. conchilega* occurs both in intertidal and subtidal soft-bottom marine areas, *Lanice conchilega* reefs and biogenic reefs in general can have a striking presence in intertidal areas (Bouma *et al.* 2009a). Furthermore, the structuring role of *L. conchilega*

reefs on the surrounding environment and the associated fauna of intertidal areas (see *The impact of* L. conchilega *on its environment*) makes *L. conchilega* an important biological species along coastlines. For this reason, and because they are the most accessible part of the marine environment, soft-bottom intertidal areas were selected as the study area of this thesis. Although coastlines have long been viewed as "deserts" (Pearse *et al.* 1942), supporting few animals and biological processes, they are interesting and generally productive ecosystems that harbour a marine fauna of great ecological diversity (Dahl 1952; McLachlan 1983). Most invertebrate phyla are represented on soft-bottom intertidal areas, either as interstitial forms or as members of the macrofauna (McLachlan and Brown 2010). Not only the sediment, but also the often neglected surf zone of intertidal areas, can sustain a rich fauna. Intertidal areas and their surf zones can function as nursery areas for early life stages of fish and other marine organisms (Beyst *et al.* 2001a; Beyst *et al.* 2001b). In terms of trophic relationships, the macrofauna of intertidal areas form part of a larger food web that includes zooplankton, fishes and birds (McLachlan and Brown 2010). Most intertidal food webs largely rely on a wide variety of sources originating from the ocean and terminate in marine end members, namely birds, fish and crabs (McLachlan and Brown 2010). Additionally, the bacterial community constitutes a main end member too, since it forms an important sink of carbon in the food webs of intertidal sediments (*e.g.* van Oevelen *et al.* 2006a; van Oevelen *et al.* 2006b). Both detritus and phytoplankton (and microphytobenthos in more sheltered intertidal areas) represent relatively constantly available food sources of marine origin (*e.g.* McLachlan and Brown 2010; Maria *et al.* 2012). Lastly, intertidal areas are of increasing concern to governmental policymakers, because of their recognized provisions of goods and services for humans, such as food provision and recreation tourism and education (Duarte 2000).

Study sites

This thesis focusses on *Lanice conchilega* reefs of temperate soft-bottom intertidal areas and includes two study sites with different environmental conditions, which enables an assessment of the generality of the results for a larger geographical area. The two

intertidal study sites are selected for their well-established *L. conchilega* reefs, and are located along the French side of the English Channel (Fig. 3).

The Bay of the Mont Saint-Michel (BMSM) is a large-scale intertidal sand flat located in the Normand-Breton Gulf (48°39.70' N – 01°37.41' W, Lower Normandy, France) (Fig. 4). The bay is intensively studied and characterized by its extreme semi-diurnal macrotidal regime with the second highest tidal range (up to 15.5 m during spring tides) in Europe (Larsonneur *et al.* 1994). As a result of the extreme tides, the intertidal zone covers up to 25000 ha of which 21000 ha of mudflats and 4000 ha of salt marshes (Lefeuvre *et al.* 2000). Due to high turbidity, phytoplankton production in the BMSM is low and primary production is assumed to be mainly based on microphytobenthos (Lefeuvre *et al.* 2000). The intertidal zone is mainly dominated by a *Macoma balthica* community characterized by low macrobenthic abundances and diversity (Retière 1979; Thorin *et al.* 2001; Trigui 2009). Species density and recruitment is much higher in the eastern part of the bay compared to the western section, due to differences in food availability, hydrology and sediment characteristics (Thorin *et al.* 2001).

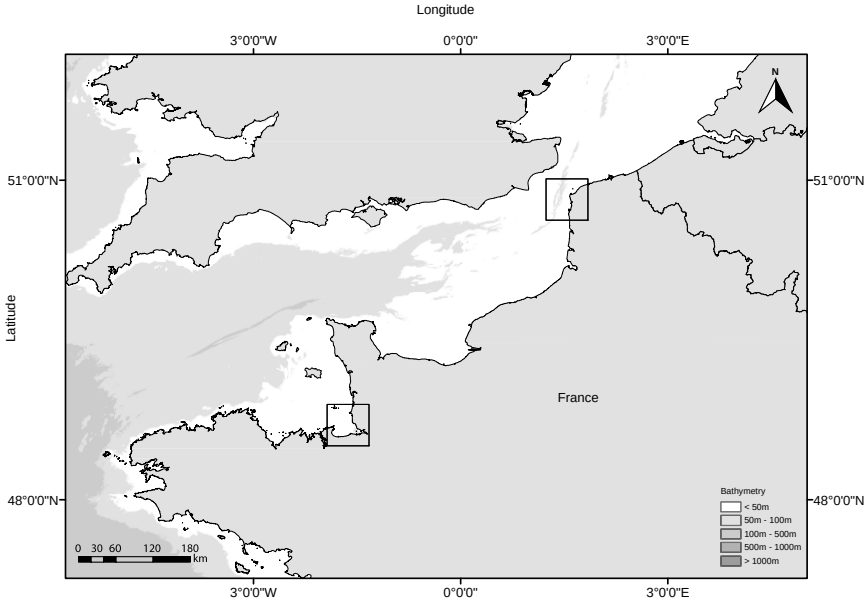


Figure 3. Study sites throughout the thesis are located along the French coast of the English Channel: Boulogne-sur-Mer (Boulogne; upper square) and the Bay of the Mont Saint-Michel (BMSM; lower square)

The bay has an important nursery function for fish species (Legendre 1984; Laffaille *et al.* 1998) and it is an internationally important migration stopover and wintering site for birds (Le Drean-Quenec'hdu *et al.* 1995), designated as a RAMSAR site and classified within the European nature conservation network 'Natura 2000' as a special protection area (SPA) under the 1979 Birds Directive[3] and a special area of conservation (SAC) under the 1992 Habitats Directive. The BMSM contains one of the largest intertidal *L. conchilega* reefs of Western Europe. Although the reef (situated in the central part of the bay and in the lower section of the tidal flat; Fig. 4) constitutes less than 1% of the total bay (190 ha in 2005), the system can be considered unique, because of its size and its rather permanent presence (Godet *et al.* 2008).

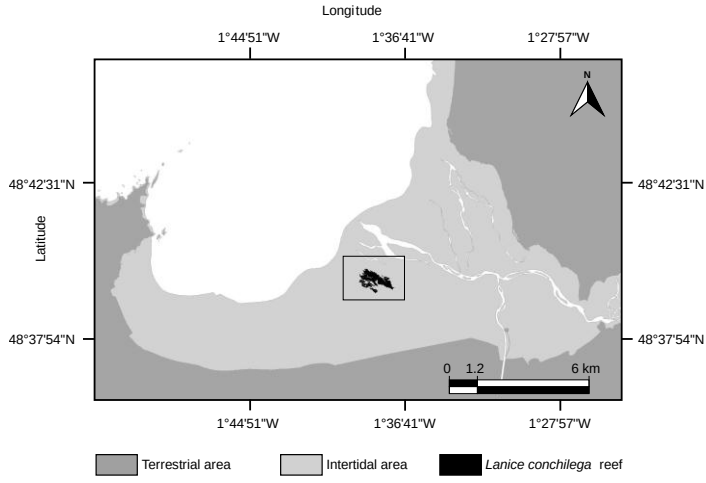


Figure 4. The Bay of the Mont Saint-Michel (BMSM, Lower Normandy, France) with the intertidal Lanice conchilega *reef located in its central part*

The site of Boulogne-sur-Mer (further referred to as Boulogne) is a soft-bottom intertidal area located along the northern part of the English Channel (50° 44.01' N – 01°35.15' E, Nord-Pas de Calais, France) (Fig. 5). Boulogne is characterized by a tidal range up to 7 m and in comparison to the BMSM, the intertidal area of Boulogne (comprising 9.43 ha of sheltered sand flat) is less pristine, sheltered by two harbour walls and much less

[3] Directive 79/409/EEG of 2 April 1979 on the Conservation of Wild Birds, *PB L* 103, 25 April 1979; http://ec.europa.eu/environment/nature/legislation/birdsdirective/index_en.htm

studied. In 2006, the *L. conchilega* reef of Boulogne could be divided into 3 reef zones: a large reef zone situated below the mean low waterline at spring tide (LWST) covering an area of about 4.5 ha, a western reef zone higher on the flat occupying about 0.4 ha, and an eastern reef zone higher on the flat occupying about 0.25 ha (Rabaut *et al.* 2008) (Fig. 5). Nevertheless, the extent of the *L. conchilega* reef areas is highly variable among seasons and years (pers. obs.).

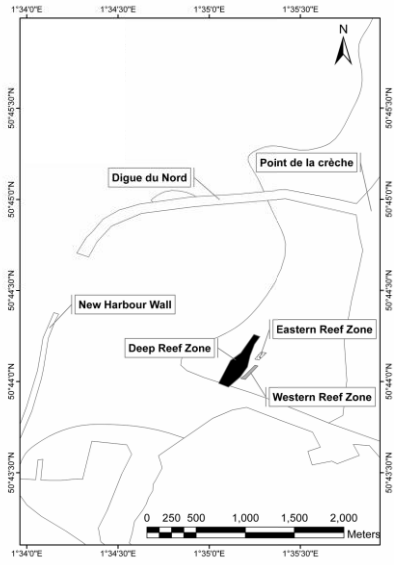


Figure 5. The intertidal area of Boulogne-sur-Mer (Nord-Pas de Calais, France) with an indication of the intertidal Lanice conchilega *reef zones of 2006 (from Rabaut et al. 2008)*

The PhD research

Objectives

Several studies have focussed on ecosystem engineers, biogenic reefs and on the polychaete *Lanice conchilega*. Nevertheless several knowledge gaps remain, especially dealing with the link between ecosystem engineers and food webs. Furthermore, many studies on ecosystem engineering have been conducted in systems in which the food web was only poorly documented. This PhD thesis aims at improving our understanding of the structural and functional role of reefs constructed by the ecosystem engineer

Lanice conchilega in natural soft-substrate coastal ecosystems. In order to study the overall importance of *L. conchilega* reefs in trophic linkages in soft-bottom intertidal areas, a holistic approach was kept in mind regarding the formulation of the ecological hypotheses throughout the thesis. At first the community and ecosystem ecology of *L. conchilega* reefs was studied, followed by characterising trophic interactions taking place within the reef habitats and comprehensively combining ecosystem engineering and trophic interactions (*i.e.* food web ecology) (Fig. 6).

The objectives of this thesis are:

Objective 1: Investigating the effect of *L. conchilega* reefs on the density, diversity and composition of the bentho-pelagic and wader community of soft-bottom intertidal areas

Objective 2: Investigating the effect of the predator *Crangon crangon* on the bioirrigation and feeding activity of an ecosystem engineer which is trophically coupled to the food web

Objective 3: Investigating ecosystem engineer-mediated effects on the structure and stability of a soft-bottom intertidal food web

Objective 4: Quantifying the carbon flows in a soft-bottom intertidal food web in the presence of ecosystem-engineered *L. conchilega* reefs

Objective 5: Assessing whether the outcomes on the structural and functional role of *L. conchilega* reefs on the ecosystem can be generalised beyond the local scale

Outline of the thesis

Apart from the general introduction and discussion, this thesis is a compilation of different research articles which have been published, submitted or are in preparation for submission to a scientific peer-reviewed journal. For that reason, the outline of the chapters resembles almost exactly the published or submitted papers. Each chapter can be read as an independent unit, which inevitably leads to an overlap between the introduction and

discussion sections of different chapters. Cited literature is compiled in the reference list at the end of this thesis. All chapters have the PhD candidate as first author.

This PhD thesis focuses on the structural and functional role of reefs constructed by the tube building polychaete *Lanice conchilega* in the overall soft-bottom intertidal food web. **Chapters 2, 3 and 5** are based on field sampling campaigns and deal with the different components of the soft-bottom intertidal food web. **Chapter 4** was founded on a laboratory based mesocosm experiment, while **Chapter 6** integrates all collected data by using linear inverse models.

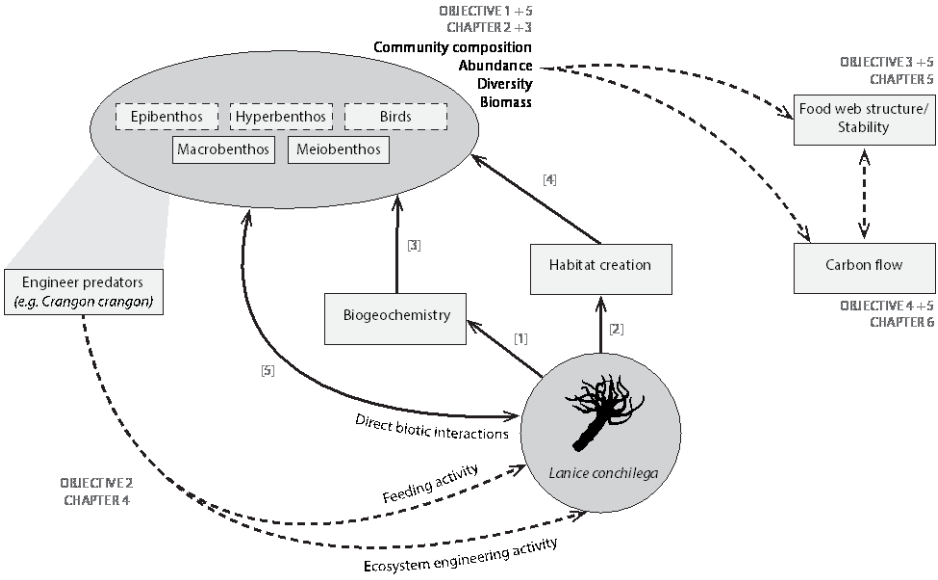


Figure 6. Schematic overview of the potential interactions involving the ecosystem engineer Lanice conchilega. *Dashed boxes and lines represent largely unknown and understudied compartments and links which will be dealt with in this PhD thesis. Full lines represent extensively studied interactions. 'Biogeochemistry' and 'Habitat creation' constitute the main environmental characteristics of the habitat affected by* L. conchilega.*[1] Forster and Graf (1995), Braeckman et al. (2010); [2] e.g. Carey (1987), Rabaut et al. (2007a), Degraer et al. (2008), Rabaut et al. (2009); [3] e.g. Zühlke et al.(1998), Braeckman et al. (2011b); [4] e.g. Carey (1987), Zühlke et al. (1998), Petersen and Exo (1999), Van Hoey et al. (2008), Godet et al. (2009), Rabaut et al. (2010), Rabaut et al. (2013); [5] e.g. Goss-Custard and Jones (1976), Yates et al. (1993), Amara et al. (2001)*

Chapter 2 discusses the effect of intertidal *L. conchilega* reefs on the bentho-pelagic components of a soft-bottom intertidal ecosystem. The different components of the benthos community (*i.e.* macro-, hyper- and epibenthos) were compared between an

area dominated by the ecosystem engineer *L. conchilega* and an area without any ecosystem engineering species. Additionally, the effect of different local environmental characteristics on the structuring role of the reef habitat was investigated. The results have been published as: *De Smet, B., D'Hondt, A.-S., Verhelst, P., Fournier, J., Godet, L., Desroy, N., Rabaut, M., Vincx, M., Vanaverbeke, J. (2015). Biogenic reefs affect multiple components of intertidal soft-bottom benthic assemblages: the* Lanice conchilega *case study. Estuarine, Coastal and Shelf Science, 152(0), 44-55.*

The study presented in **Chapter 3** quantifies the role of the *L. conchilega* reef of the Bay of the Mont Saint-Michel as potential "oases" for feeding waders. For the first time, a combination of macrobenthos data, bird counts and bird diet information based on faecal analysis was used. The findings were compared with literature data in order to stress the importance of the reef habitats. This chapter has been published as: *De Smet, B., Godet, L., Fournier, J., Desroy, N., Jaffré, M., Vincx, M., Rabaut, M. (2013). Feeding grounds for waders in the Bay of the Mont Saint-Michel (France): the* Lanice conchilega *reef serves as an oasis in the tidal flats. Marine biology, 160(4), 751-761*. Parts of this chapter result from the master thesis dissertation of the PhD candidate (2011) entitled "*Intertidal* Lanice conchilega *reefs as feeding grounds for wading birds in the Bay of the Mont Saint-Michel (France)*".

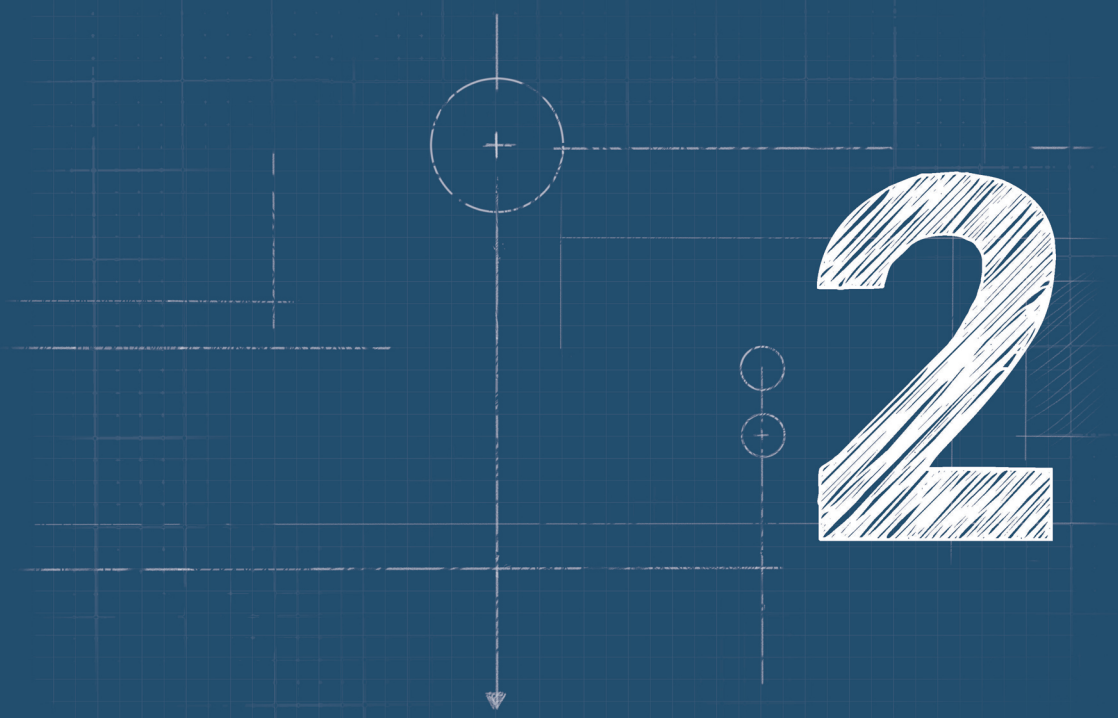
Chapter 4 discusses the existence of positive or negative feedback effects of species impacted by the ecosystem engineering activities of *L. conchilega*. An experimental laboratory approach was used to investigate the effects of predation pressure on the activity of an intertidal *L. conchilega reef* by measuring the bioirrigation and food uptake of *L. conchilega* in the reef. This paper has been submitted to *Journal of experimental marine biology and ecology* as: *De Smet, B., Braeckman, U., Soetaert, K., Vincx, M., Vanaverbeke, J. (under review). Predator effects on the feeding and bioirrigation activity of ecosystem-engineered* Lanice conchilega *reefs.*

In **Chapter 5**, the potential of an ecosystem engineer to modify the structure and dynamics of a food web was studied. A hypothesis linking ecosystem engineering to food webs was investigated based on a field sampling approach of intertidal biogenic reefs created by the ecosystem engineer *L. conchilega*. To this end, a classical and a more

integrative approach based on stable isotope data of primary food sources and the bentho-pelagic consumer community of an intertidal *L. conchilega* reef and control area were used. This paper has been accepted for publication in *PLoS ONE* as: *De Smet, B., Fournier, J., De Troch, M., Vincx, M., Vanaverbeke, J. (in press). Integrating Ecosystem Engineering and Food Web Ecology: Testing the Effect of Biogenic Reefs on the Food Web of a Soft-Bottom Intertidal Area.*

All data collected in the previous chapters is integrated in **Chapter 6**, which makes use of linear inverse models (LIMs) to present a comprehensive analysis of carbon cycling and dynamics of an intertidal soft-bottom food web dominated by biogenic reefs. The aim of this study is to quantify the bentho-pelagic food web both in the presence and absence of the ecosystem engineer *L. conchilega*, with a focus on the macrofaunal subweb. In order to address temporal and spatial variation in the food web structure, two study sites and two time periods were taken into account. This chapter is in preparation for publication as: *De Smet, B., van Oevelen, D., Vincx, M., Vanaverbeke, J., Soetaert, K.* Lanice conchilega *structures carbon flows in soft-bottom intertidal areas.*

Finally, **Chapter 7** (General Discussion, Conclusions and Future Challenges) discusses the results from this PhD thesis in a broader perspective and ends with future prospectives for the understanding of the ecological role of *L. conchilega* reefs and biogenic reefs in general.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Chapter 2

Biogenic reefs affect multiple components of intertidal soft-bottom benthic assemblages: the *Lanice conchilega* case study

Slightly modified from the publication:

De Smet, B., D'Hondt, A.-S., Verhelst, P., Fournier, J., Godet, L., Desroy, N., Rabaut, M., Vincx, M., Vanaverbeke, J. (2015). Biogenic reefs affect multiple components of intertidal soft-bottom benthic assemblages: the *Lanice conchilega* case study. Estuarine, Coastal and Shelf Science, 152(0), 44-55.

Abstract

Biogenic reefs composed of the tube-building polychaete *Lanice conchilega* are important from a conservation point of view because they noticeably increase the biodiversity in otherwise species poor environments. However, up to now, little or no attention has been paid to the intertidal epi- and hyperbenthic communities associated with the reefs. Therefore, this is the first study which focuses on the effect of *L. conchilega* reefs on the entire bentho-pelagic community at two different locations. Biotic and abiotic environmental variables were measured and macro-, epi-, and hyperbenthic communities were sampled within a *L. conchilega* reef and a control area at two locations in France: the Bay of the Mont Saint-Michel (BMSM) and Boulogne-sur-Mer (Boulogne). The effect of the reef presence on the benthic community was studied with a 3-factor (Reef, Location and Period) Permanova. In addition, the relationship between the benthic community and the environmental variables was investigated using Distance-based linear models (DistLM). The majority of the collected organisms was sampled in the reef area (macrobenthos: 91 %, epibenthos: 81 % and hyperbenthos: 78.5%) indicating that, independent of the location, the *L. conchilega* reefs positively affect all three associated benthic communities. However, the extent of the effect seems to be most pronounced for the macrobenthos and less distinct in case of the hyperbenthos. The macro- and epibenthos are mainly structured by *L. conchilega* density and macrobenthic food availability respectively, while the hyperbenthos is rather structured by biotic environmental variables. In general, *L. conchilega* reefs do not only affect abundances and diversity but also the presence and relative contribution of species belonging to multiple trophic levels.

Key words: benthos; tidal flats; *Lanice conchilega*; community composition; biogenic reefs; intertidal area

Introduction

Numerous organisms are widely known to modify their environments and influence other species. Recently, there has been a growing interest in biota that directly or indirectly modulate the availability of resources (other than themselves) to other species, by causing physical state changes in biotic or abiotic materials; so-called ecosystem engineers (Jones *et al.* 1994). Typically, ecosystem engineers are classified as either *autogenic* (changing the environment via their own physical structures; *e.g.* corals) or *allogenic* (changing the environment by transforming living or non-living materials from one physical state to another; *e.g.* woodpeckers, beavers) (Jones *et al.* 1994). In the marine environment, ecosystem engineers are known to strongly modify coastal sediments in temperate, tropical and semi-tropical locations (Kirtley and Tanner 1968; Micheletti-Flores and Negreiros-Fransozo 1999; Bouma *et al.* 2009a) and possibly create persistent emergent structures referred to as 'biogenic reefs' (Carey 1987; Rabaut *et al.* 2009; Callaway *et al.* 2010; Godet *et al.* 2011). Seagrass fields (*e.g.* Bouma *et al.* 2009b), bivalve accumulations (*e.g.* Gutiérrez *et al.* 2003) and polychaete tube assemblages (*e.g.* Dubois *et al.* 2002; Chapman *et al.* 2012) are some of the most striking biogenic structures composed of ecosystem engineers in coastal environments. A prime example is the aggregations composed of the Sandmason *Lanice conchilega* (Polychaeta, Terebellidae). This species may achieve densities of several thousands of individuals per square meter (Van Hoey *et al.* 2006) and as such generate elevated sediment reefs (Rabaut *et al.* 2009). *L. conchilega* displays both allogenic (*e.g.* by providing oxygen supply) and autogenic (*e.g.* by sedimentological alterations) ecosystem engineering properties (Godet *et al.* 2008; Rabaut *et al.* 2009) and is therefore bound to receive a higher ecosystem engineer qualification (Braeckman *et al.* 2014). Hence, this species is considered an ideal model organism for studying the sediment-animal-interactions in a modified habitat (Reise *et al.* 2009). The habitat modifications of this ecosystem engineer result in an increased habitat complexity and heterogeneity, facilitating the evolution of a more diverse and abundant macrobenthic community (Rabaut *et al.* 2007a; Toupoint *et al.* 2008).

Biogenic reefs, including *L. conchilega* reefs, are listed as Habitat 1170 under Annex I of the EC Habitats Directive EEC/92/43, and are hence important from a conservation point of view (Godet *et al.* 2008; Rabaut *et al.* 2009). Nevertheless, research on the structural and functional role of biogenic reefs, and ecosystem engineers in general, mostly focuses on one single ecosystem component at a time (*i.e.* macrobenthos, Rabaut *et al.* (2007a); or fish, Rabaut *et al.* (2010)) and takes place on the local scale (*i.e.* the Belgian part of the North Sea for Rabaut *et al.* (2007a), Rabaut *et al.* (2010) and Rabaut *et al.* (2013)). To proceed towards the widespread implementation of conservation measures for biogenic reefs, understanding the general ecological function of the reefs, including their simultaneous effect on multiple ecological components and possible associated food web interactions, is of crucial importance. Additionally, in order to avoid merely local evidence on the importance of the reefs, a generalization of the outcome, and hence conducting research beyond the local scale, should be aimed at. In the case of *L. conchilega,* the impact of a reef is most elaborately studied regarding the associated macrobenthic community (Zühlke 2001; Rabaut *et al.* 2007a; Van Hoey *et al.* 2008; De Smet *et al.* 2013). Furthermore, some top-down effects on meiofauna (Zühlke *et al.* 1998; Braeckman *et al.* 2011b) and biogeochemical characteristics (Braeckman *et al.* 2010; Passarelli *et al.* 2012); and bottom-up effects on juvenile flatfish (Rabaut *et al.* 2010; Rabaut *et al.* 2013) and waders (Petersen and Exo 1999; De Smet *et al.* 2013) have been reported. All this suggests that *L. conchilega* reefs play a central role in the link between benthic, pelagic and air-borne parts of the intertidal food web. Nonetheless, rather little or no attention has been paid to the entire intertidal epibenthic (benthic organisms living on the surface of the sediment, such as most crabs, shrimp and starfish; Baretta-Bekker *et al.* 1992) and hyperbenthic community (small animals living in the water layer close to the seabed; Mees and Jones 1997) associated with *L. conchilega* reefs.

This study took into account the entire range of bentho-pelagic components forming part of the intertidal *L. conchilega* reef and exceeded the local scale, by incorporating two different locations along the French coast characterized by different environmental settings. The following null hypotheses were tested: (1) the *L. conchilega* reef does not affect the different components of the benthos community (*i.e.* macro-, epi- and

hyperbenthos); and (2) the reef effect, if any, is not affected by the local environmental characteristics of the reef area.

Material & Methods

Study area

The two sampling locations were selected based on the presence of a well-established intertidal *L. conchilega* reef and are located along the French side of the English Channel: (1) the Bay of the Mont Saint-Michel (BMSM), a large-scale intertidal sand flat located in the Normand-Breton Gulf (48°39.70' N - 01°37.41' W; Lower Normandy, France); and (2) Boulogne-sur-Mer (further referred to as Boulogne), a soft-bottom intertidal area along the northern part of the English Channel (50°44.01'N - 01°35.15'E; Northern France). The main *L. conchilega* reef of the BMSM is situated in the central part of the bay and in the lower section of the tidal flats. The large tidal flats, covering 25 000 ha, result from the extreme megatidal regime (tidal range up to 15.5 m during spring tides) which dominates the BMSM (Larsonneur *et al.* 1994). Boulogne is characterized by a tidal range up to 7 m and in comparison to the BMSM, the intertidal area of Boulogne is less pristine and sheltered by two harbour walls (Rabaut *et al.* 2008). The *L. conchilega* reef is situated in the lower intertidal and the majority is only exposed during extreme mean low water spring tide conditions.

Sampling design, sampling and laboratory treatment

Sampling took place in 2012, during spring (from 7th till 13th of March in the BMSM and from 22nd till 25th of March in Boulogne) and autumn (from 17th till 21st of September in BMSM and from 15th till 18th of October in Boulogne). To cover an adequate amount of reef heterogeneity, a *L. conchilega* reef area of 100x100 m was defined at each location. In addition, a second 100x100 m sampling area (control), in the absence of any bioengineering species was defined. The bathymetric level between the reefs and their respective control areas was similar and the sampling areas were at least 300 m apart. At every sampling area, several components of the soft-bottom assemblage were sampled simultaneously: water, sediment, macro-, epi- and hyperbenthos.

Three water samples were taken during ebbing tide for the determination of suspended particulate matter (SPM, $mg.L^{-1}$) and chlorophyll a (Chl *a*, $\mu g.L^{-1}$) concentrations. For both SPM and Chl *a*, an appropriate subsample was filtered onto precombusted (450°C for 2h) and pre-weighed Whatman GF/F filters (47 mm) and subsequently stored at -80°C until analysis. Filters for Chl *a* were lyophilised and pigments were extracted in 90% acetone. Chl *a* concentrations of the supernatant were determined using HPLC (Gilson) analysis (Wright and Jeffrey 1997).

Within each sampling area, ten sediment samples were randomly taken during low tide with a core (Ø 3.6 cm) for sedimentologic factors (median grain size and mud content) and the total amount of organic matter (TOM). Sediment grain size was analysed by means of a Malvern Mastersizer 2000; TOM was determined per sample by weighing the difference between the dry weight (48h by 60°C) and the weight after 2h by 500°C. The Chl *a* concentration ($\mu g.g^{-1}$ dry sediment) of the upper sediment layer was determined using HPLC (Gilson) analysis (as described above) and used as a proxy for the microphytobenthos (MPB) biomass (Jeffrey *et al.* 1997). *L. conchilega* densities were estimated by counting the tube tops with fringes on photographs of ten randomly placed metal frames (0.25 m^2) (Ropert and Dauvin 2000; Van Hoey *et al.* 2006). Finally, per sampling area, ten macrobenthos samples were collected with an inox corer (Ø 12 cm, 40 cm deep), sieved through a 1-mm circular mesh size and fixed with a neutralized 8% formalin solution. In the laboratory, samples were rinsed and preserved using a neutralized 4% formalin solution with 0.01% Rose Bengal until processing. All macrobenthos was sorted, counted and identified to the lowest possible taxonomic level.

The lower water column (up to 40 cm) covering the sampling areas was sampled to study the epi- and hyperbenthic communities of the *L. conchilega* reef. Epibenthos was sampled with a 2 m beam trawl during daytime ebbing tide. The net was 3 m long, had a mesh size of 9x9 mm and was equipped with a tickler-chain in the ground rope. Similarly, hyperbenthos was collected with a hyperbenthic sledge during daytime ebbing tide. The sledge consisted of a metal frame (100x40 cm) and was equipped with two identical nets: a lower and an upper net. Both nets were 3 m long and (at the

mouth) 20 cm high with a mesh size of 1x1mm. The epi- and hyperbenthic devices were towed in the surf zone across the defined sampling area and parallel to the coastline for 100 m. In Boulogne, they were pulled by two persons, while in the BMSM a zodiac was used at a speed of one knot, due to the extreme tides and the extent of the bay. At least three epibenthos and three hyperbenthos replicates (trawls) were taken at each sampling area. Catches were fixed in a neutralized 8% formalin solution. In the laboratory, samples were stained with 0.01% Rose Bengal, rinsed, sorted and identified to the lowest possible taxonomic level. Because of the large content of three hyperbenthos Boulogne autumn samples, subsamples (20% of the total sample weight) were taken.

Data analysis

Environmental variables

Since the assumptions of parametric statistical approaches were not fulfilled for none of the environmental variables (water SPM and Chl *a* concentrations; sediment grain size, mud content and TOM; MPB biomass), the effect of the presence of a *L. conchilega* reef on the selected environmental variables was explored with a permutational ANOVA (Permanova) in which Reef (reef versus control), Location (BMSM versus Boulogne) and Period (spring versus autumn) were fixed factors. The analysis was based on an Euclidean distance resemblance matrix and performed on untransformed data; except for TOM which was fourth-root transformed in order to meet homogeneity of dispersions (Anderson *et al.* 2008). In case a significant effect was found, pair-wise tests among all pairs of levels of the given factor(s) were carried out. Although Permanova assumes no explicit assumptions regarding the distributions of the original variables, a test for the homogeneity of multivariate dispersions was ran, using the PERMDISP routine. Factors were considered significant at $p < 0.05$ in all analyses. For mud content, MPB biomass, water SPM and Chl *a* concentrations the PERMDISP test was significant for some factors (even after transformation), indicating differences in dispersion. Therefore, prudence is advised when interpreting the results and the relative sizes of the within and between-group resemblances deserve further attention (Anderson *et al.* 2008).

Macro-, epi- and hyperbenthic community descriptors

Faunistic terms are used here as work definitions based on the efficiency of the sampling gear, following Beyst *et al.* (2001a; 2001b), resulting in a semi-artificial classification. For the macrobenthos, *L. conchilega* itself was excluded; as well as strictly hyper- or epibenthic organisms (Appendix 1). The remaining abundance data was standardised per m^2. For the hyperbenthos, strictly macrobenthic, larger epibenthic and sessile organisms were removed from the dataset (Appendix 2), while for the epibenthos, strictly macrobenthos and animals which were more efficiently caught with the hyperbenthic sledge (small sized crustaceans such as isopods and mysids; early postlarval fish and jellyfish) were excluded (Appendix 3). Different developmental stages of decapods (zoea, megalopa and juveniles) were treated as different 'taxa', since they have a different ecology (Beyst *et al.* 2001a). Epi- and hyperbenthic abundances were standardized per 1000 m^2. Univariate Permanova analyses were based on Euclidean distance resemblance matrices and performed on untransformed data; except for macrobenthic species abundance and epibenthic species richness which were fourth-root transformed in order to meet homogeneity of dispersions (Anderson *et al.* 2008). Prior to Permanova, analysis of similarity (one-way ANOSIM) based on Bray-Curtis resemblance matrices of untransformed data was used to test for (dis-)similarities in the hyperbenthic community between the lower and upper net. The same 3-factor Permanova design as for the environmental variables was used on the macro-, hyper- and epibenthic community descriptors (species abundance *N*, species richness *S*, Shannon-Wiener diversity index *H'* and Pielou's evenness index *J'*). In case a significant effect was found, pair-wise tests were carried out. Since our main interest is the effect of a *L. conchilega* reef on the associated communities, only significant results including the factor Reef are shown. All PERMDISP tests were significant for one or more factors, except for macrobenthic species abundance and *H'*; epibenthic species richness, *J'* and *H'*; and hyperbenthic species richness, species abundance and *J'*. Multivariate 3-factor Permanova analyses (see univariate analysis) were based on Bray-Curtis resemblance matrices of fourth-root transformed macro-, epi- and hyperbenthos community abundance data. According to a PERMDISP test, the Reef x Period and the Reef x Location interactions for the macrobenthic community and the factor Reef for the

epibenthic community showed significant differences in dispersion. In addition, data were visualised by a Principal Coordinates Analysis (PCO) (Anderson *et al.* 2008). Based on Spearman correlations, only species of the macro-, epi- and hyperbenthic community that correlated > 50 % with one of the first 2 PCO axes were plotted. Distance-based linear models (DistLM) were carried out to investigate the relationship between the benthic community and the biotic and abiotic environmental variables (Anderson *et al.* 2008). For the macrobenthic DistLM, all environmental variables (grain size, mud content, TOM, MPB, Chl *a* and SPM) and one biotic predictor variable (*L. conchilega* density) were used. In comparison to the macrobenthic community, 3 additional biotic predictor variables (macrobenthic *N*, *S* and *H')* were used for the epi- and hyperbenthic DistLMs. Macro-, epi- and hyperbenthic abundance data was fourth-root transformed and a Bray-Curtis resemblance matrix was used. If necessary, environmental and biotic predictor variables were square-root or log transformed to avoid skewness. Variables were tested for multi-collinearity and redundant variables were removed from the analysis in case the correlation $|r| \geq 0.95$ (Anderson *et al.* 2008). A significance level of $p < 0.05$ was used in all tests. All statistical analyses were performed within PRIMERv6 with the PERMANOVA+ add-on software (Clarke and Gorely 2006; Anderson *et al.* 2008).

Results

Environmental variables

Fine (125 – 250 µm) and medium (250 – 500 µm) sand were the major sediment fractions in all samples. Median grain size in the BMSM ranged from 196 to 324 µm, while in Boulogne from 185 to 261 µm (Table 1). A significant Reef x Location x Period effect for average median grain size was revealed (3-factor Permanova: pseudo-F = 5.27, $p = 0.026$; Appendix 4). However, differences due to Reef were only significant in the BMSM (pair-wise tests: reef < control in spring: $p = 0.0001$; and reef < control in autumn $p = 0.0002$). The average mud content (fractions < 63 µm; Table 1) was significantly affected by the Reef x Location interaction (3-factor Permanova: Pseudo-F = 11.80, $p = 0.0001$; Appendix 4). Pairwise tests showed a significant difference between the reef and control area of the BMSM (reef > control: $p = 0.0001$). Mean TOM content (Table 1) was

shown to be significantly different due to the factors Reef (Permanova: Pseudo-F = 17.41, p = 0.0001; reef > control; Appendix 4), Location (Permanova: Pseudo-F = 55.94, p = 0.0001; BMSM > Boulogne; Appendix 4) and Period (Permanova: Pseudo-F = 6.44, p = 0.0081; spring > autumn; Appendix 4).

Table 1. Median grain size (n = 10), mud content (n = 10), total organic matter (n = 10), microphytobenthos (MPB) biomass (n = 10), SPM (n = 3) and Chl a (n = 3) (mean ± SE) for different combinations of Reef (reef vs. control), Location (BMSM vs. Boulogne) and Period (spring vs. autumn).

	BMSM				Boulogne			
	Spring		Autumn		Spring		Autumn	
	Reef	Control	Reef	Control	Reef	Control	Reef	Control
Median grain size (µm)	228 ± 5	285 ± 6	234 ± 8	315 ± 5	221 ± 6	231 ± 5	227 ± 5	226 ± 3
Mud content (%)	6.66 ± 2.74	0 ± 0	5.3 ± 1.24	0 ± 0	0.86 ± 0.59	0 ± 0	0.44 ± 0.44	0 ± 0
Total organic matter (%)	1.67 ± 0.17	1.13 ± 0.04	1.5 ± 0.14	1 ± 0.08	1.4 ± 0.55	0.53 ± 0.04	0.59 ± 0.08	0.52 ± 0.03
MPB (µg.g^{-1} sediment)	1.68 ± 0.25	0.74 ± 0.06	1.55 ± 0.21	1.77 ± 0.82	5.84 ± 1.51	1.31 ± 0.26	0.89 ± 0.12	0.46 ± 0.03
SPM (mg.L^{-1} water)	770.46 ± 66.69	953 ± 60.8	98 ± 2.04	256 ± 7	257.45 ± 36.25	172.98 ± 5.6	184.5 ± 4.09	182.83 ± 1.48
Chl*a* (µg.L^{-1} water)	49.13 ± 2.76	45.45 ± 11.16	3.56 ± 0.24	6.53 ± 0.68	43.15 ± 2.52	21.6 ± 0.7	3.73 ± 0.06	3.3 ± 0.37

Mean microphytobenthos (MPB) biomass of the upper sediment layer (Table 1) was significantly affected by the interactions of Reef x Location (3-factor Permanova: Pseudo-F = 5.67, p = 0.0163; Appendix 4), and Reef x Period (3-factor Permanova: Pseudo-F = 8.74, p = 0.0025; Appendix 4). A pair-wise test for the Reef x Location interaction showed a significantly higher MPB biomass for the reef vs. control area of Boulogne (p = 0.0014) but not for the BMSM, while a pair-wise test for the Reef x Period interaction showed a significantly higher MPB biomass for the reef vs. control area in spring (p = 0.0007), but not in autumn. A 3-factor Permanova revealed a significant difference in mean SPM (Table 1) for Reef x Location (Pseudo-F = 16.86, p = 0.0012; Appendix 4), resulting from a significant difference between the reef and control area both in the BMSM (pair-wise test: reef > control: p = 0.0163) and Boulogne (pair-wise test: reef < control: p = 0.03). Finally, regardless of the location, the mean Chl *a* concentration of the water was observed to be substantially higher in spring samples compared to autumn samples (Table 1). The mean Chl *a* concentration was significantly affected by the Reef x Period interaction (3-factor Permanova: Pseudo-F = 4.88, p = 0.0375; Appendix 4), as a result of a significant difference in autumn (pair-wise test: reef < control: p = 0.0096), and was significantly higher in the BMSM (3-factor Permanova; Location: Pseudo-F = 6.85, p = 0.01).

Effect of reef and location on the macrobenthic community

In total, 100 910 macrobenthic organisms belonging to 57 different taxa were sampled in the BMSM; of which 83 637 organisms (belonging to 44 taxa) in the reef area and 17 273 organisms (belonging to 34 taxa) in the control area. 62% of the taxa overlapped between the reef and control area. In Boulogne, 299 185 macrobenthic organisms were sampled (of which 294 548 in the reef area and 4 727 in the control area), belonging to 58 taxa (53 taxa in the reef area and 16 taxa in the control area; with 17% of the taxa overlapping). Mean *L. conchilega* density (± SE) in the BMSM reef was 1 724 ± 292 ind.m^{-2} and 5 044 ± 589 ind.m^{-2} in the Boulogne reef. Excluding *L. conchilega*, 96% of the remaining macrobenthos was classified in three major taxa: polychaetes (61%), amphipods (23.4%) and bivalves (15.6%). The macrobenthic community in the BMSM was dominated by the bivalves *Cerastoderma edule* (39.5%) and *Macoma balthica* (13.3%), and the polychaete *Malmgreniella arenicolae* (6.8%). In Boulogne, the amphipod genus *Urothoe* (26%) and the polychaetes *Eumida sanguinea* (19.1%), *Pygospio elegans* (16.3%), *Heteromastus filiformis* (13.5%) and *Capitella sp.* (11.6%) were dominant.

Table 2. Overview of L. conchilega *densities and of the calculated community descriptors (mean ± SE) for the macrobenthic, epibenthic and hyperbenthic communities of the* L. conchilega *reef and control area of the Bay of the Mont Saint-Michel (BMSM) and Boulogne-sur-Mer.*

	BMSM				Boulogne			
	Spring		Autumn		Spring		Autumn	
Macrobenthos	Reef (n = 10)	Control (n = 10)	Reef (n = 10)	Control (n = 10)	Reef (n = 10)	Control (n = 10)	Reef (n = 10)	Control (n = 10)
L. conchilega density	1090 ± 269	0 ± 0	2358 ± 444	0 ± 0	6720 ± 868	0 ± 0	3368 ± 296	0 ± 0
Species abundance N	4964 ± 478	800 ± 239	3400 ± 529	927 ± 225	13446 ± 1708	209 ± 45	16009 ± 3192	255 ± 45
Species richness S	11.0 ± 1.1	4.9 ± 0.8	10.1 ± 1.0	6.2 ± 1.1	16.0 ± 0.9	2.0 ± 0.4	14.9 ± 1.0	2.0 ± 0.3
Shannon diversity index H	1.555 ± 0.123	1.341 ± 0.149	1.775 ± 0.129	1.546 ± 0.155	1.829 ± 0.120	0.509 ± 0.267	1.714 ± 0.103	0.569 ± 0.165
Pielou's eveness index J'	0.655 ± 0.032	0.925 ± 0.023	0.778 ± 0.031	0.930 ± 0.022	0.665 ± 0.047	0.976 ± 0.016	0.638 ± 0.029	0.966 ± 0.017
Epibenthos	Reef (n = 3)	Control (n = 4)	Reef (n = 3)	Control (n = 3)	Reef (n = 3)	Control (n = 3)	Reef (n = 4)	Control (n = 4)
Species abundance N	487 ± 111	155 ± 44	755 ± 421	406 ± 190	1452 ± 674	205 ± 128	9912 ± 2983	353 ± 83
Species richness S	9.3 ± 0.7	5.8 ± 0.9	8.3 ± 1.5	10.7 ± 3.4	5.7 ± 0.7	3.0 ± 0.6	9.0 ± 0.0	9.0 ± 1.2
Shannon diversity index H	1.176 ± 0.108	1.284 ± 0.112	1.186 ± 0.097	1.316 ± 0.245	0.855 ± 0.225	0.500 ± 0.159	0.547 ± 0.219	1.463 ± 0.107
Pielou's eveness index J'	0.532 ± 0.068	0.757 ± 0.069	0.568 ± 0.006	0.594 ± 0.012	0.509 ± 0.153	0.450 ± 0.063	0.249 ± 0.100	0.674 ± 0.027
Hyperbenthos	Reef (n = 3)	Control (n = 3)	Reef (n = 3)	Control (n = 3)	Reef (n = 3)	Control (n = 4)	Reef (n = 3)	Control (n = 3)
Species abundance N	3350 ± 1360	5363 ± 1832	27853 ± 14977	8107 ± 153	23323 ± 9219	2965 ± 1015	7357 ± 5087	450 ± 217
Species richness S	20.3 ± 3.5	21.0 ± 3.0	38.3 ± 2.9	38.7 ± 3.5	1.0 ± 4.0	14.7 ± 1.4	19.0 ± 4.5	11.7 ± 5.6
Shannon diversity index H	1.864 ± 0.175	1.662 ± 0.119	1.306 ± 0.438	2.361 ± 0.159	1.015 ± 0.167	1.482 ± 0.305	1.477 ± 0.323	1.413 ± 0.713
Pielou's eveness index J'	0.638 ± 0.101	0.549 ± 0.031	0.358 ± 0.116	0.646 ± 0.028	0.365 ± 0.042	0.555 ± 0.116	0.526 ± 0.142	0.752 ± 0.008

Permanova revealed that all community descriptors (Table 2) were significantly affected by the Reef x Location interaction (Appendix 5). Pair-wise comparisons revealed a significantly higher macrobenthic abundance (Fig. 1) and species richness, and a significantly lower J' in reef areas compared to control areas, both in the BMSM and

Boulogne (for all pair-wise tests: p = 0.0001). Although significant differences between the reef and control area were detected for *H'* in Boulogne (pair-wise test: reef > control: p = 0.0001), none could be detected for the BMSM (pair-wise test: p = 0.1249). Seasonal variances of all calculated indices were negligible in comparison to variances as a result of Reef and/or Location, except for *L. conchilega* density.

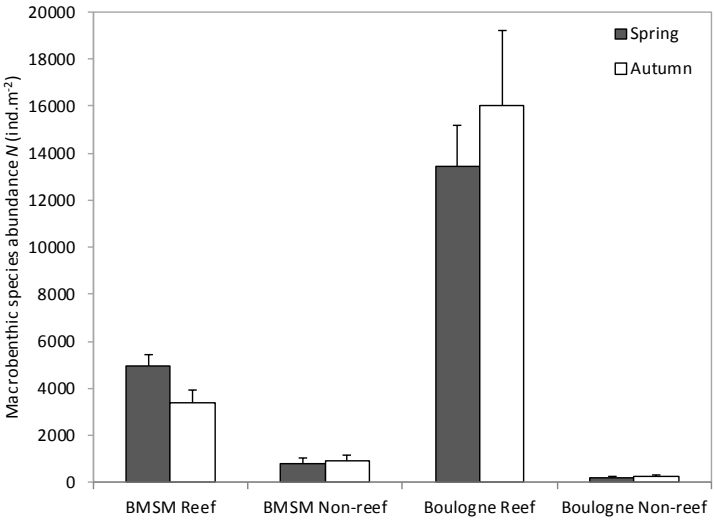

Figure 1. Mean macrobenthic species abundance (excluding L. conchilega*) N (± SE) per m² for different combinations of Reef (reef vs. control), Location (BMSM vs. Boulogne) and Period (spring vs. autumn)*

Multivariate analyses revealed a significant interaction effect of Reef x Location (3-factor Permanova: Pseudo-F = 14.49, p = 0.0001) and Reef x Period (3-factor Permanova: Pseudo-F = 3.40, p = 0.0041) on the macrobenthic community. Differences between the *L. conchilega* reef and control areas were highly significant for the two locations and the two periods (all four pair-wise tests: p = 0.0001). The PCO analysis (Fig. 2) showed a clear separation of reef samples vs. control samples. Moreover, the reef areas of the BMSM and Boulogne were clearly distinguishable from one another, while this was less the case for the control areas. PCO axis 1 explained 30.2 % of the total variation inherent in the resemblance matrix and separated reef samples and control samples. Besides, reef areas were characterized by a larger array of taxa compared to areas in the absence of *L. conchilega*. PCO axis 2 explained 17.9 % of the total variation and distinguished the

locations, albeit not clearly for the control areas. The DistLM analysis showed that the variables *L. conchilega* density (15.1 %), mud content (9.0 %) and median grain size (4.4 %) together explain 28.45 % of the variation in the macrobenthic community structure (Appendix 6).

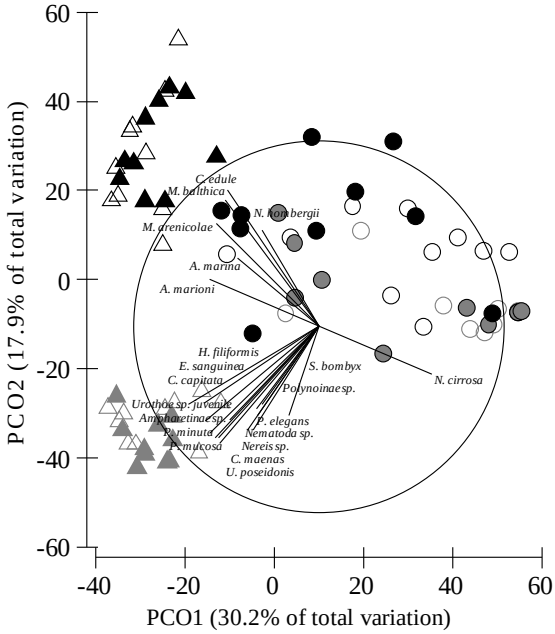

Figure 2. PCO analysis of macrobenthos communities in reef and control areas in the BMSM and Boulogne during spring and autumn 2012 based on Bray-Curtis similarities of fourth-root transformed abundance data. Vectors represent species correlating > 50% (based on Spearman correlation coefficients) with one of the first two PCO axes (▲ Reef/BMSM/Spring; △ Reef/BMSM/Autumn; Reef/Boulogne/Spring; △ Reef/Boulogne/Autumn; ● Control/BMSM/Spring; ○ Control/BMSM/Autumn; ● Control/Boulogne/Spring; ○ Control/Boulogne/Autumn)

Effect of reef and location on the epibenthic community

In the BMSM, 5 565 organisms belonging to 29 different taxa were sampled. The majority was sampled in the reef area (3 725 organisms; 21 taxa) in comparison to the control area (1 840 organisms; 24 taxa). 55% of the taxa in the BMSM overlapped between the reef and control area. The Boulogne sampling yielded 46 030 organisms (23 taxa), of which 44 005 organisms (17 taxa) were caught in the reef area and 2 025 organisms (16 taxa) in the control area. 39% of the taxa in Boulogne overlapped

between the reef and control area. 94.6% of the epibenthos was confined to three taxonomic groups: *Crangon crangon* (89.8%), *Pomatoschistus spp.* (9%) and flatfish species (1.2%). The epibenthic community in the BMSM reef and control area was mainly dominated by the brown shrimp *Crangon crangon* (54.6% & 57.6% resp.) and by the gobies *Pomatoschistus lozanoi* (14.6% & 13.6% resp.) and *Pomatoschistus microps* (18.8% & 3% resp.). *Crangon crangon* was dominant in the epibenthic community of the Boulogne reef (90.5%) and control area (43.7%). In the latter area its dominance was complemented by the common shore-crab *Carcinus maenas* (23.5%) and *Pomatoschistus minutus* (21%). Species richness (Table 2) differed significantly between various combinations of Reef x Period (3-factor Permanova: Pseudo-F = 5.93, $p = 0.0243$), which is the result of a significantly higher species richness in the reef vs. control area during spring (pair-wise test: $p = 0.0049$). Mean epibenthic abundance (Fig. 3), *J'* and *H'* (Table 2) were significantly affected by the Reef x Location x Period interaction (Appendix 5).

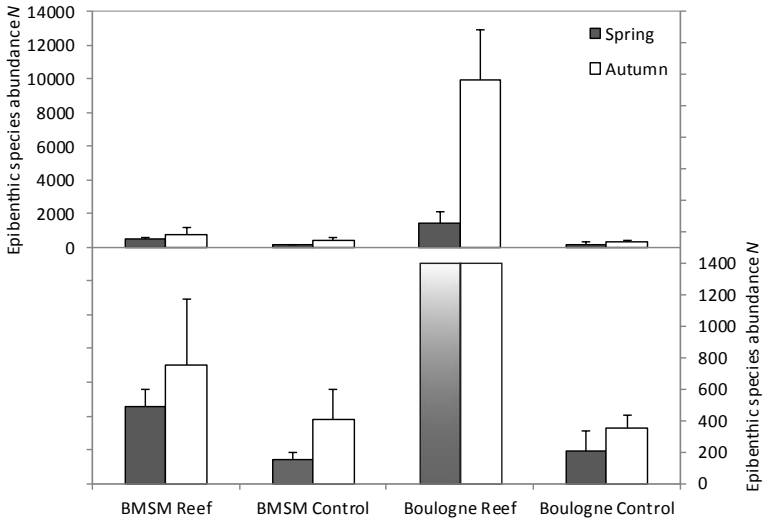

Figure 3. Mean epibenthic species abundance N (± SE) per 1000m² for different combinations of Reef (reef vs. control), Location (BMSM vs. Boulogne) and Period (spring vs. autumn). An overall abundance view is given in the upper panel, while the lower panel shows a more detailed view on the abundances of BMSM - Reef, BMSM - Control and Boulogne - Control

In case of the mean epibenthic abundance, the significant effect was due to significant differences between the reef and control areas in the BMSM during spring (pair-wise test: reef > control: $p = 0.032$) and in Boulogne during autumn (pair-wise test: reef >

control: $p = 0.0283$). For both *J'* and *H'*, the significant differences between the reef and control areas resulted from differences in Boulogne during autumn (pair-wise test *J'*: reef < control: $p = 0.0273$; pair-wise test *H'*: reef < control: $p = 0.0284$).

Multivariate analyses demonstrated that the epibenthic community differed among the Reef x Period interaction (3-factor Permanova: Pseudo-F = 2.36, $p = 0.016$). Pair-wise tests showed that the epibenthic communities of the *L. conchilega* reef area and the control area differed both in spring ($p = 0.0139$) and autumn ($p = 0.0016$). Simultaneously, Permanova revealed highly significant community differences between the locations (*Location*: Pseudo-F = 17.56, $p = 0.0001$). According to the PCO analysis, PCO axis 1 mainly showed a distinction between locations (explaining 34.6 % of the total variation), while PCO axis 2 separated spring samples and autumn samples (explaining 20.6 % of the total variation) (Fig. 4). The DistLM analysis showed that the variables SPM (17 %), macrobenthic *H'* (11.8 %), macrobenthic *N* (21 %) and Chl *a* (9.8 %) together explain 59.6 % of the variation in the epibenthic community structure (Appendix 6).

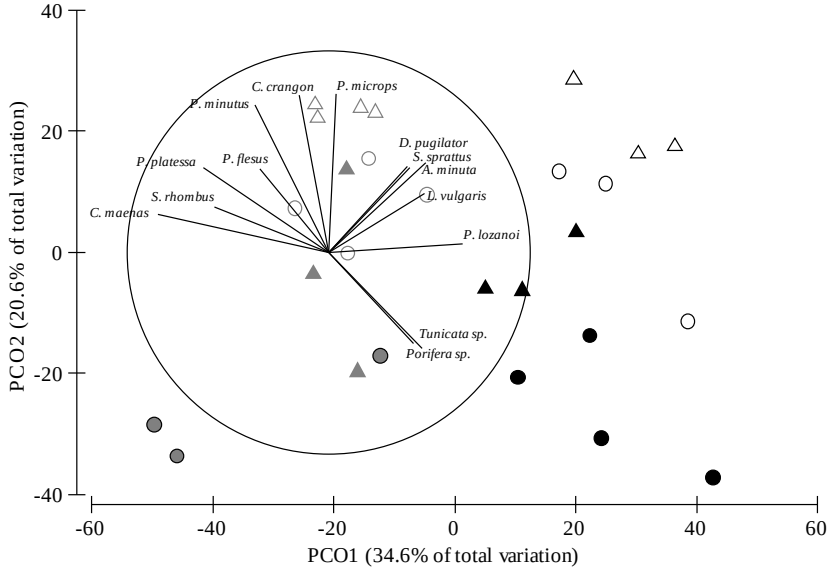

Figure 4. PCO analysis of epibenthos communities in reef and control areas in the BMSM and Boulogne during spring and autumn 2012 based on Bray-Curtis similarities of fourth-root transformed abundance data. Vectors represent species correlating > 50% (based on Spearman correlation coefficients) with one of the first two PCO axes (▲ Reef/BMSM/Spring; △ Reef/BMSM/Autumn; ▲ Reef/Boulogne/Spring; △ Reef/Boulogne/Autumn; ● Control/BMSM/Spring; ○ Control/BMSM/Autumn; ● Control/Boulogne/Spring; ○ Control/Boulogne/Autumn)

Effect of reef and location on the hyperbenthic community

In the BMSM, 134 020 organisms belonging to 100 different taxa were sampled. The majority was sampled in the reef area (93 610 organisms; 74 taxa) in comparison to the control area (40 410 organisms; 72 taxa) and 44% of the taxa overlapped between the reef and control area. In Boulogne, 105 250 organisms belonging to 72 taxa were caught, of which 92 040 organisms (56 taxa) in the reef area and 13 210 (41 taxa) in the control area; with 32% of the taxa overlapping between the reef and control area. 98.2% of the hyperbenthos could be classified in 5 taxonomic groups: mysidae (61.3%), shrimp (15.5%), amphipoda (13.9%), juvenile fish (6.9%) and juvenile crab (2.4%). The hyperbenthic community of the BMSM was mainly dominated by mysid shrimp: *Mesopodopsis slabberi* (72.3%) in the reef area; and *Schistomysis kervillei* (20.2%), *M. slabberi* (18.1%), *Schistomysis spiritus* (13%) and Mysidae sp. (12.7%) in the control area. In the Boulogne reef area, the hyperbenthic community was dominated by *Mesopodopsis slabberi* (27.5%), *Crangon crangon* juveniles (24.2%) and the amphipod *Nototropis swammerdamei* (17.9%); while dominated by sandeel *Ammodytes tobianus* juveniles (48.7%), *Crangon crangon* juveniles (16.1%) and Pleuronectiformes sp. juveniles (10.2%) in the control area. The hyperbenthic community was not significantly different between the lower and upper nets (one-way ANOSIM: $p = 0.543$; R = 0.008). Consequently, further statistical analyses were done excluding the factor Net. A 3-factor Permanova revealed that hyperbenthic abundance (Fig. 5) and *J'* (Table 2) are significantly affected by Reef (*N*: Pseudo-F = 6.48, reef > control: $p = 0.0107$; *J'*: Pseudo-F = 4.93, reef < control: $p = 0.0421$; Appendix 5), while species richness (Table 2) was affected by Location (Pseudo-F = 30.11, BMSM > Boulogne: $p = 0.0001$; Appendix 5) and Period (Pseudo-F = 11.52, spring < autumn: $p = 0.0048$; Appendix 5). *H'* (Table 2) did not differ significantly for any of the factorial interactions.

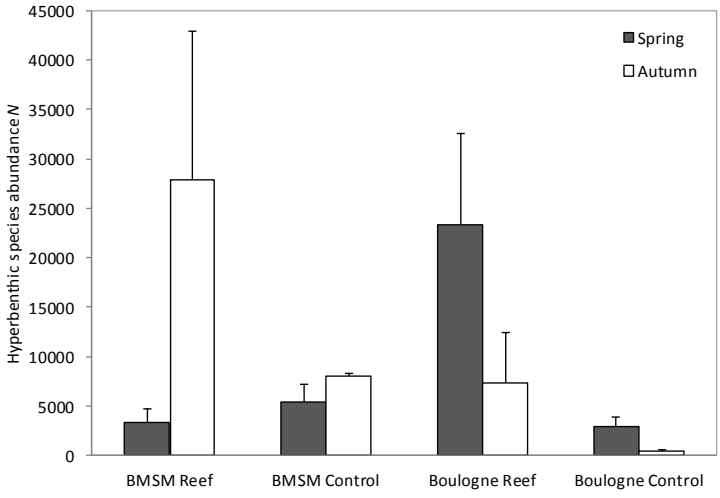

Figure 5. Mean hyperbenthic species abundance N (± SE) per 1000m² for different combinations of Reef (reef vs. control), Location (BMSM vs. Boulogne) and Period (spring vs. autumn)

Multivariate analyses revealed that the Reef x Location x Period interaction affects the hyperbenthic community (3-factor Permanova: Pseudo-F = 2.14, p = 0.0339). However, only in Boulogne during spring the hyperbenthic community seemed to be significantly different between the *L. conchilega* reef and the control area (pair-wise test: p = 0.0139). According to the PCO analysis, PCO axis 1 explained 27.3 % of the total variation; distinguishing samples from the BMSM and Boulogne (Fig. 6). Moreover, the hyperbenthic community of the BMSM was characterized by a diverse species composition compared to Boulogne. PCO axis 2 explained 20 % of the total variation and separated spring and autumn samples, which was more pronounced for Boulogne. The DistLM analysis showed that the variables TOM (16.1 %), Chl *a* (15.5 %), SPM (18.6 %) and grain size (8.2 %) together explain 58.5 % of the variation in the hyperbenthic community structure (Appendix 6).

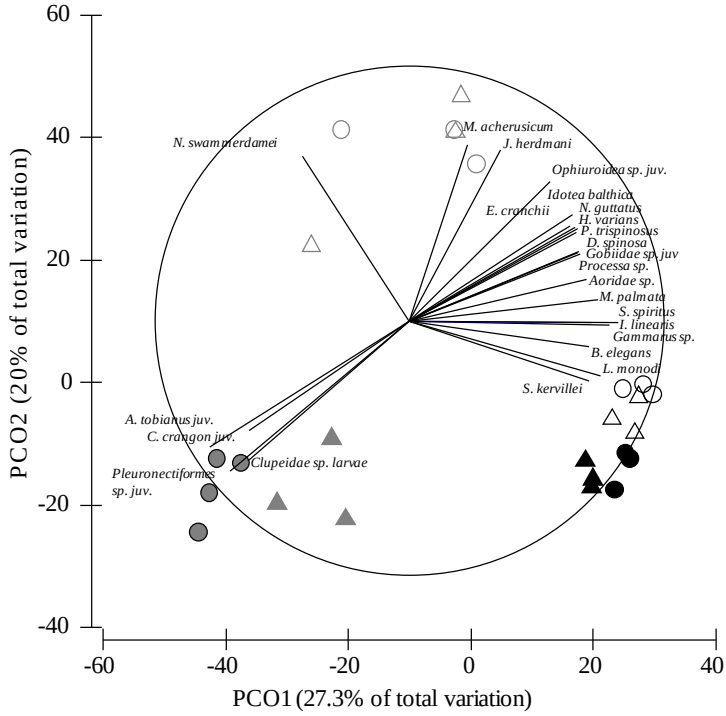

Figure 6. PCO analysis of hyperbenthos communities in reef and control areas in the BMSM and Boulogne during spring and autumn 2012 based on Bray-Curtis similarities of fourth-root transformed abundance data. Vectors represent species correlating > 50% (based on Spearman correlation coefficients) with one of the first two PCO axes (▲ Reef/BMSM/Spring; △ Reef/BMSM/Autumn; ▲ Reef/Boulogne/Spring; △ Reef/Boulogne/Autumn; ● Control/BMSM/Spring; ○ Control/BMSM/Autumn; ● Control/Boulogne/Spring; ○ Control/Boulogne/Autumn)

Discussion

Lanice conchilega reef effect on the macro-, epi- and hyperbenthic community

This study shows that the presence of *L. conchilega* reefs affects the associated macro-, epi- and hyperbenthic communities. For all three investigated communities, the majority of the collected organisms was sampled in the reef (macrobenthos: 91 %; epibenthos: 81 %; hyperbenthos: 78.5 %), showing a more abundant benthic reef community compared to bare sands. Based on the proportion of animals in the reef versus control areas, the extent of the structuring effect seems to be most pronounced for the macrobenthos and least strong for the hyperbenthos. We suggest that this is due to the differential dependency of the three communities to the sea floor. The link

between the benthic community and the sediment, where the effects of *L. conchilega* are most pronounced, is much more intimate for the macro- and epibenthos than for the hyperbenthos (Fig. 7). Moreover, the reefs predominantly affect the macrobenthic part of the benthos, which corroborates previous observations (Zühlke *et al.* 1998; Rabaut *et al.* 2007a; Van Hoey *et al.* 2008; De Smet *et al.* 2013). The increased macrobenthic abundance, species richness and diversity is attributed to an increased habitat heterogeneity and the increased provision in shelter/refuge and primary food sources accomplished by the high tubeworm density. Similarly, epibenthic animals, which are considered to be free-living and highly mobile species in soft bottom assemblages, seem to select for *L. conchilega* reefs. Their mobile character enables them to actively move to and feed upon the macrobenthos within the biogenic habitat. As shown by the epibenthic DistLM analysis, the increased macrobenthic diversity (*H'*) and abundance (*N*) in the reef are in favor of the epibenthic community, as demonstrated before for *e.g.* (flat)fish (Rabaut *et al.* 2013). The macrobenthic reef community in this study is characterized by a large array of taxa, of which bivalves (*Cerastoderma edule* and *Macoma balthica*), polychaetes (*Eumida sanguinea*) and amphipods (*Urothoe poseidonis*) are most abundant (Fig. 7). The positive effect of macrobenthos on higher trophic levels is in accordance with preliminary results of trophic relationships within *L. conchilega* reefs based on stable isotope and stomach content analysis (De Smet *et al.* unpublished). Similarly, polychaetes, amphipods and other small benthic animals are counted as a non-negligible part of the diet of macrocrustaceans such as the omnivorous brown shrimp, *Crangon crangon* (Boddeke *et al.* 1986; Oh *et al.* 2001). Abundances of this epibenthos species in the reef areas largely exceeded those from bare sand patches. In general, *C. crangon* is one of the most abundant epibenthic species in the European intertidal zones (Hostens 2000; Beyst *et al.* 2001b) and supports a large commercial fishery in northern European waters (Temming and Damm 2002). Hence, by fueling stocks of commercial fish species, *C. crangon* might be one of the most important inhabitants of the *L. conchilega* reef. Besides shrimp, *Pomatoschistus spp.* and flatfish species were notably represented in the reefs (Fig. 7). Several (commercial) benthic fish species were already shown to be attracted to biogenic habitats because

they provide preferred prey species and/or act as refuges against predation (Kaiser *et al.* 1999; Rabaut *et al.* 2010; Chapman *et al.* 2012).

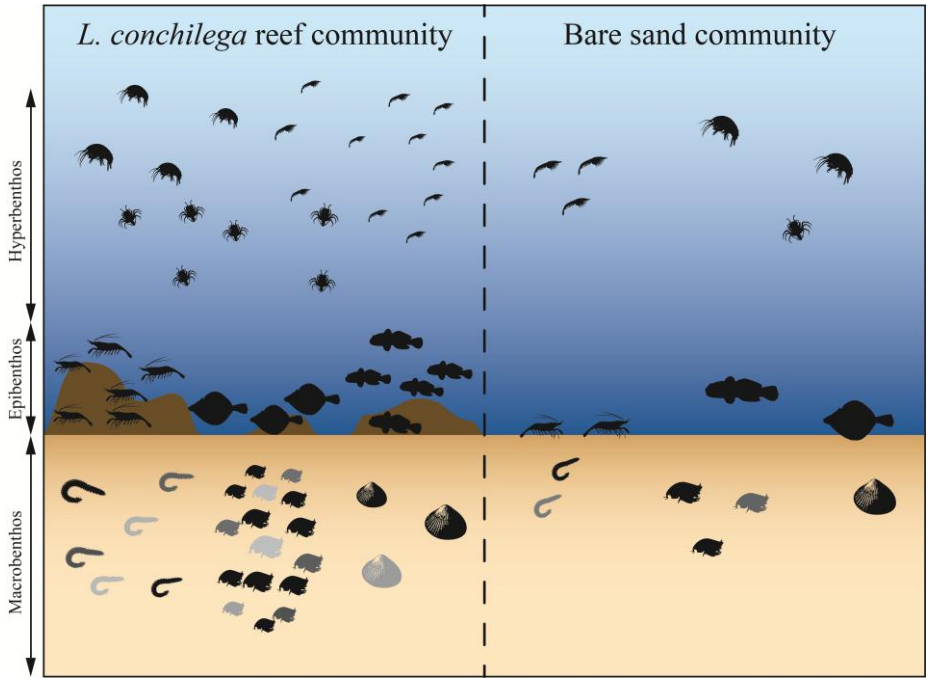

Figure 7. Schematic overview of the effect of the L. conchilega *reef (left) and a bare sand habitat (right) on the macro-, epi- and hyperbenthic communities of a soft-bottom intertidal food-web. Differential dependency of the benthic communities to the sea floor account for differences in the extent of the structuring effect of the* L. conchilega *reef in terms of abundances (number of symbols) and species diversity (different shades of grey). Macrobenthos: polychaetes (e.g.* Eumida sanguinea*), amphipods (e.g.* Urothoe poseidonis*) and bivalves (e.g.* Cerastoderma edule*); Epibenthos:* Crangon crangon*, Pomatoschistus sp., flatfish sp. (*Pleuronectes platessa*); Hyperbenthos: mysida (*Mesopodopsis slabberi*), amphipoda (*Nototropis swammerdamei*) and decapod megalopa larvae*

Hyperbenthic communities were affected by the presence of the *L. conchilega* reefs. However, neither *L. conchilega* density nor macrobenthic species richness (*S*), abundance (*N*) or diversity (*H'*) do contribute to the DistLM models explaining variation in the hyperbenthic assemblages. This suggests that the structuring effect of the reefs is indirect for the hyperbenthos. The high hyperbenthos abundances (dominated by mysids and to a lesser extent amphipods, juvenile fish and decapods, and shrimp excluding *C. crangon*) in the *L. conchilega* assemblages may be partly explained by their active migration to favorable environments (Dewicke *et al.* 2002). Highly motile

hyperbenthos often reaches high densities in regions with a strong input of organic matter (Dauvin *et al.* 1994; Mees and Jones 1997). Therefore, the elevated amounts of TOM in the reefs, resulting from changes in the hydrodynamic regime due to the presence of *L. conchilega* tubes, can be assumed to sustain the high hyperbenthic abundances observed in our study. The increased *L. conchilega* density, and hence the shelter against predation provided by the tubes, seems to be of a lesser importance to attract hyperbenthic animals. Notwithstanding their active, behavior-mediated transport, the hyperbenthos is subjected to passive transport mechanisms as well (Mees and Jones 1997; Dewicke *et al.* 2002), most probably explaining their limited reef selectivity.

In general, the macro- and epibenthic communities are largely structured by the tubeworm density and macrobenthic food availability respectively, while the hyperbenthos, which is the least dependent on the sea bed, is rather structured by biotic environmental variables.

The effect of the local environment on the macro-, epi- and hyperbenthic community

At both locations, the macrobenthic reef samples are heavily clustered, which demonstrates that although the reef areas are very patchy, their species composition remains the same on a local spatial scale. On a larger scale, the magnitude of the reef effect on the macro-, epi- and hyperbenthos seems to be influenced by the location of the reef. For the macro- and epibenthos, the differences in the community descriptors between a reef and control area were more pronounced for Boulogne than for the BMSM (except for the epibenthic species richness). The location effect is most likely driven by the difference in *L. conchilega* density between both sites, as shown by the DistLM analyses. The higher tubeworm densities (± SE) in Boulogne (5 044 ± 589 ind.m^{-2}) compared to the BMSM (1 724 ± 292 ind.m^{-2}) can provide a larger and more secure settlement surface of larval and postlarval benthic organisms (Qian *et al.* 1999; Rabaut *et al.* 2007a). Being characterized by a higher macrofaunal abundance and species diversity, high density *L. conchilega* reefs act as feeding grounds for more mobile epibenthic animals. Nevertheless, the high density reef of Boulogne was observed to

have a lower mud content (silt + clay) and lower amounts of TOM compared to the lower density reef of the BMSM. This finding is in contradiction to the higher macrofaunal abundances in the Boulogne reef, whose growth is believed to be promoted by the sedimentation of organic matter (Wieking and Kröncke 2005). Although the *L. conchilega* density is the most explaining predictor variable, the location effect is amplified by other location dependent environmental variables (*e.g.* grain size and Chl *a*).

Differences in the hyperbenthic community between locations were less pronounced and not attributable to the density of the tubeworm, but rather to environmental variables inherent to the location such as TOM, Chl *a* and SPM. Therefore, at the local scale of the intertidal flat, the hyperbenthos, which is dominated by mysids and known to feed mainly on detritus, algae and zooplankton (Mauchline 1980), is largely structured by the availability of food.

The presence of the *L. conchilega* reef undoubtedly affects the abundances of all three investigated benthic communities and most notably the macrobenthic component. Moreover, our study shows that intertidal *L. conchilega* reefs do not only affect abundances and diversity but also the presence and relative contribution of species belonging to multiple trophic levels. As such, an altered predator-prey relationship in *L. conchilega* reefs versus bare sands can be presumed. Furthermore, the positive feedback of *L. conchilega* reefs on higher trophic levels of intertidal areas is shown to be applicable on locations with different environmental conditions. This outcome supports the current idea that *L. conchilega* reefs are important habitats and might be crucial towards the implementation of widespread conservation measures in the future.

Acknowledgements

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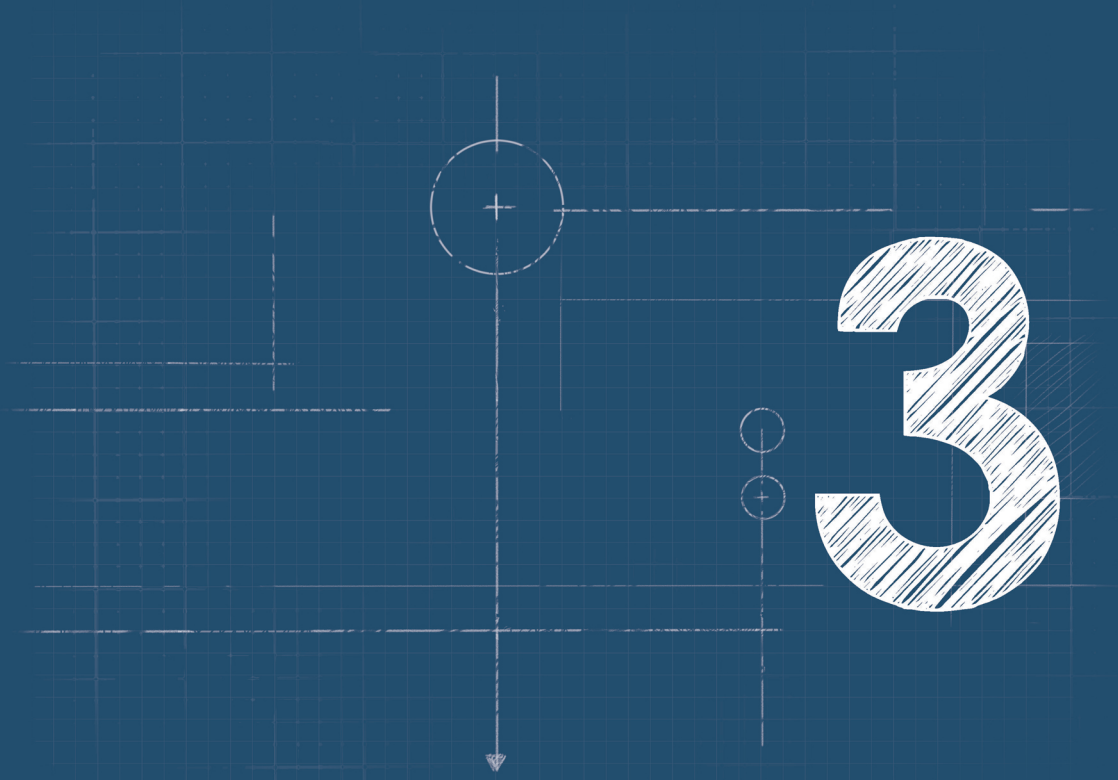
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Chapter 3

Feeding grounds for waders in the Bay of the Mont Saint-Michel (France): the *Lanice conchilega* reef serves as an oasis in the tidal flats

Slightly modified from the publication:

De Smet, B., Godet, L., Fournier, J., Desroy, N., Jaffré, M., Vincx, M., Rabaut, M. (2013). Feeding grounds for waders in the Bay of the Mont Saint-Michel (France): the *Lanice conchilega* reef serves as an oasis in the tidal flats. Marine biology, 160(4), 751-761.

Abstract

The tube-building polychaete *Lanice conchilega* can form dense populations, often called reefs, which enhance the density and diversity of the benthic community, therefore constituting feeding grounds for secondary consumers. The aim of this study was to quantify the role of the *L. conchilega* reef of the Bay of the Mont Saint-Michel (BMSM) for feeding waders, by combining macrobenthos data, bird counts and bird diet information. Wader densities in the reef were on average 46.6 times higher than in non-reef areas. According to faecal analyses, waders in the reef mainly selected the accompanying fauna and especially crustaceans. The attractiveness of the reef to feeding birds may be largely explained by the high abundance, richness and biomass of macrobenthic species in the reef compared to the rest of the BMSM.

Key words: *Lanice conchilega*, waders, Charadriiformes, reef, feeding grounds, food consumption, faecal analysis.

Introduction

Lanice conchilega is a widespread tubicolous polychaete that can form dense aggregations, considered biogenic reefs (Rabaut *et al.* 2009; Callaway *et al.* 2010). Above particular density thresholds, the structures of the tubes as well as the biological activity of the "engineer species" (Callaway 2006; Godet *et al.* 2008), generate specific sedimentological "structures" (Carey 1987; Feral 1989) and enhance the species diversity and abundance of the associated benthic macrofauna by stabilizing the sediments (Zühlke 2001; Callaway 2006; Rabaut *et al.* 2007a; Van Hoey *et al.* 2008). These reefs are also important feeding grounds for flatfishes, particularly the juveniles of *Pleuronectes platessa* (Rijnsdorp and Vingerhoed 2001; Rabaut *et al.* 2010).

Lanice conchilega can be an important item in the diet of several waders (Goss-Custard and Jones 1976; Yates *et al.* 1993). Godet *et al.* (2009) showed that oystercatchers may significantly select *L. conchilega* reefs for feeding and that their spatial distribution can change greatly when these reefs disappear. But to our knowledge, the study of Petersen and Exo (1999) in the German part of the Wadden Sea is, so far, the only extensive study investigating the role of large *L. conchilega* dominated tidal flats for waders and gulls. In comparison to the surrounding area, they found higher densities of four bird species feeding on these flats, though the relative biomass consumption on these flats was similar to other regions of the Wadden Sea. Furthermore, the study revealed that gulls tend to feed on *L. conchilega* specimens, while waders rather select the accompanying benthic macrofauna.

The area surveyed by Petersen and Exo (1999) was special because of the spatial dominance of *L. conchilega* sand flats (60% of the 6 680 ha of the study site), and because the remaining area was covered by two other habitat-creating species: *Arenicola marina* (20%) and *Mytilus edulis* (5 to 10%). Despite of the dominance of *L. conchilega*, the tubeworm aggregates did not generate the specific sedimentological structures, typically mounds and shallow depressions, previously described for other *L. conchilega* reefs (Carey 1987).

The aim of this current study was to quantify the role of *L. conchilega* reefs as potential "oases" for feeding waders; do the reefs constitute a localized and discrete habitat for birds among large and homogeneous tidal flats? Therefore, one of the largest intertidal *L. conchilega* reefs in Europe, located in the Bay of the Mont Saint-Michel (BMSM) (France), was selected. For the first time, a combination of macrobenthos data, bird counts and bird diet information was used to determine the importance of a *Lanice conchilega* reef for feeding waders. The following hypotheses have been tested: i) the benthic macrofaunal composition of the reef clearly differs from the macrofaunal composition of the whole bay; ii) the abundance and composition of waders on the reef is significantly different from the wader abundances and composition at the scale of the whole BMSM. Additionally, the diet of waders feeding on the reef was determined by means of a faecal analysis.

Material and Methods

Study area

The *L. conchilega* reef is located in the central region of the BMSM (48°40'45" N-01°41'25" W, south-eastern part of the Normand-Breton Gulf, France) (Figure 1) and in the lower section of the tidal flats. In 2008, the reef covered 105 ha; *i.e.* 0.42% of the sand flats of the BMSM (Godet *et al.* 2011) (Figure 1). The BMSM is subjected to an extreme megatidal regime (tidal range up to 15.5 m during spring tides), resulting in large tidal flats - covering 25 000 ha - and mainly dominated by a *Macoma balthica* community characterized by low macrobenthic abundances and diversity (Retière 1979; Thorin *et al.* 2001). The BMSM is an internationally important migration stopover and wintering site for birds (Le Drean-Quenec'hdu *et al.* 1995), designated as a RAMSAR site and classified as a Special Protection Area (SPA) and a Special Area of Conservation (SAC). More than 50 000 waders winter in the BMSM; 12% of the French wintering abundances of waders (Le Drean-Quenec'hdu *et al.* 1998). At this site, Dunlin *Calidris alpina*, Red Knot *Calidris canutus*, Oystercatcher *Haematopus ostralegus*, Grey Plover *Pluvialis squatarola*, Black-

tailed Godwit *Limosa limosa*, and Bar-tailed Godwit *Limosa lapponica* reach international abundance levels[4] in winter (Deceuninck and Maheo 2000).

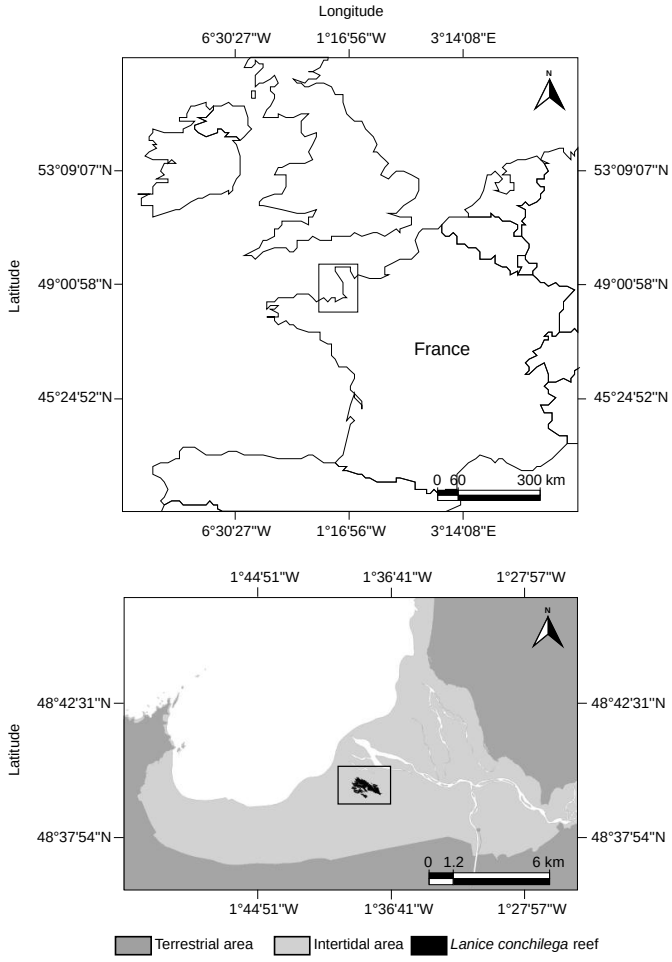

Figure 1. Location of the Bay of the Mont Saint-Michel (France) and the Lanice conchilega *reef within the bay*

Macrobenthos sampling and treatment at the scale of the *L. conchilega* reef

Benthic macrofauna was sampled from the 10th to the 12th of January 2009 within 1 ha squares of a regular grid (consisting of 150 squares). The *L. conchilega* density within

[4] Thresholds set by BirdLife International: BirdLife International (2000). *Important bird areas in Europe: priority sites for conservation. Cambridge, UK (Conservation Series No. 8)*, BirdLife International (2004). *Birds in Europe: population estimates, trends and conservation status. Cambridge, UK (Conservation Series No. 12).*

each square was estimated by counting aboveground tubes on pictures of 3 randomly selected 1/4 m^2 quadrats. Samples were only taken in one out of every two squares of each row of the grid, though every square with *L. conchilega* densities ≥ 200 ind. m^{-2} was sampled as well (*i.e.* 80 sampled squares in total) (Figure 2). The number of tubes is highly correlated with the number of individuals in the sediment (*e.g.* Van Hoey *et al.* 2006). At every selected square, one macrofaunal core was collected (1/40 m^2, 30 cm deep). Benthic samples were sieved in the field through a 1 mm circular mesh size and the retained biological material was immediately preserved in a 4.5% buffered formalin solution. In the laboratory, samples were sorted and macrobenthos was identified to the lowest possible taxonomic level. Total biomass was estimated by determining the dry mass of all individuals per species (60 °C for 48 h). The ash-free dry mass (AFDM) was calculated as the difference between the dry mass and the ash mass (500 °C for 3 h).

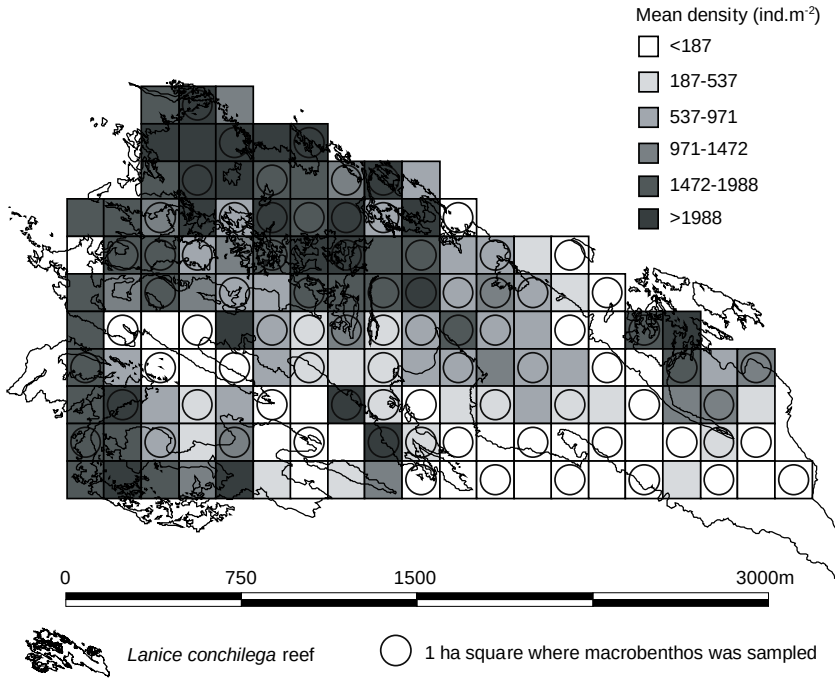

Figure 2. Macrobenthic sampling design and mean Lanice conchilega *densities on the* L. conchilega *reef in the Bay of the Mont Saint-Michel (BMSM) from 2005 to 2008. Macrobenthic samples were taken at alternating squares of each row of the grid (consisting of 150 squares). In addition, every square with a mean* L. conchilega *density ≥ 200 ind. m^{-2} was sampled as well, resulting in 80 sampled squares in total*

Wader counts at the scale of the BMSM

To assess the total number of waders in the whole BMSM, all waders of the site were monitored 5 times (January '09, March '09, May '09, September '09 and January '10) by 10 to 30 people. A standardized protocol to monitor water birds of the BMSM, developed by two ornithological associations, Bretagne-Vivante/SEPNB and GONm, was followed (Beaufils *et al.* 2009). Waders were counted in their high-tide roosts by people equipped with telescopes and binoculars. Because of the extent of the BMSM, it was divided in sectors assigned to one or two observers. To avoid double counts, surveys in the different sectors were performed on the same day and during the same time interval (20 to 30 min). Additionally, any bird group seen flying from one sector to another was systematically reported with the exact time and flight direction.

Wader counts at the scale of the reef

On the reef, birds were surveyed 21 times from February 2009 to January 2010 (at least once each month, except for November and December), but only while spring tide fully exposed the reef (*i.e.* corresponding to a low tide level of less than 2.5 m above extreme low water spring tide). Birds were counted by two persons equipped with a pair of binoculars and a telescope (magnification respectively 10X and 20-60X). All individuals were counted (in case of a few tens of individuals) or estimated in tens of individuals (if several hundreds or thousands of individuals were present). The observation point, located on a sandbank just outside the study area, ensured visibility of the entire site as well as a minimal bird disturbance.

Estimating the diet of waders feeding on the reef: faeces sampling and treatment

In 2010, faeces of five wader species were collected within the reef: Oystercatcher *H. ostralegus*, Dunlin *C. alpina*, Curlew *Numenius arquata*, Grey Plover *P. squatarola* and Bar-tailed Godwit *L. lapponica*. We selected these species because they: i) are abundant on the reef; ii) feed regularly on the reef; iii) are species for which faeces are easy to collect because they feed in dense and virtually monospecific groups. Faeces of Curlew and Dunlin were collected on the 27th of April 2010, while faeces of Oystercatcher, Bar-tailed

Godwit and Grey Plover were collected on the 13th of August 2010, the 9th of September 2010 and the 7th of October 2010, respectively.

Prior to collection, a large monospecific flock of birds feeding on the reef was observed in order to be confident that a dropping came from the target species. After 20 to 30 min (in order to be sure that the collected faeces resulted from a feeding activity on the reef) (Cramp and Simmons 1983), the entire droppings were scraped off the surface and preserved in 70% ethanol. In the laboratory, the ethanol was removed by pouring the dropping onto a 20 μm sieve. For analysis, the sample was transferred to a 100 ml jar, containing a mix of 80% distilled water and 20% hydrogen peroxide (H_2O_2), for a period of at least 24 h. The samples were shaken regularly and, after sedimentation, the supernatant was poured through a 20 μm sieve. The supernatant remaining on the sieve was transferred to a 25 cm^3 petri dish in order to observe and identify lighter animal parts (*e.g.* polychaete chaetae) with an inverted microscope. A stereo-microscope was used to screen the entire settled sand fraction for hard remnants of bivalves, polychaete jaws, etc. Prior to investigation, we selected for each of the five bird species 15 faecal samples (except for Curlew: 11 samples).

Although all different items in a faecal sample were quantified, identification to genus or species level was not always possible. Therefore, each unique unidentified animal part was assigned to a morphotype, leading up to the creation of a catalogue consisting of pictures[5]. Finally, for several analyses, morphotypes were pooled into taxonomic groups, as mentioned hereafter.

Data and statistical analysis

Macrobenthic density, richness, diversity and production in the reef

Macrobenthic densities (D), species richness (S) and species diversity (H', Shannon Index, Shannon 1948) were calculated. Abundances of macrobenthos in the *L. conchilega* reef were evaluated by summing the numbers of individuals from the different squares.

[5] De Smet B.; Godet L.; Fournier J.; Desroy N.; Jaffré M.; Vinx M.; Rabaut M.; Marine Biology Research Group - Ugent, Belgium; (2013): Picture catalogue of morphotypes found in faecal samples of wading birds in the *Lanice conchilega* reef of the Bay of the Mont Saint-Michel (France). http://dx.doi.org/10.14284/37

Subsequently, relative abundances were calculated. The annual macrobenthic production in the reef was estimated using an empirical model based on biomass and abundance data (Brey 1999; Brey 2001). The model takes additional data on benthic taxa and environmental variables such as bathymetry and temperature in consideration. Prior to production estimation, benthic biomass in g of AFDM was converted to kJ via conversion factors for aquatic organisms (Brey 2001; Brey *et al.* 2010).

Abundances and community composition of waders on the reef versus the entire BMSM

The community composition of waders was analysed with the PRIMER v6 statistical package (Clarke and Warwick 1994). Analysis of similarity (one-way ANOSIM) was used to describe (dis-)similarities in wader communities between the entire bay and the *L. conchilega* reef. Data were standardized (in order to eliminate the abundance effect) and square root transformed before conducting the analysis. To test whether waders significantly selected the reef at low tide, we compared the abundances of waders present on the reef with predicted wader abundances present on the reef assuming a homogeneous distribution of birds across the entire BMSM during low tide. Because the ratio of the area covered by the reef to the area of the tidal flats is 1:238 (105 ha reef among 25 000 ha of tidal flats), counting n individuals of a species at high tide in the whole BMSM results in a predicted abundance of n/238 individuals of this species on the reef. Only counts which were performed at similar time periods over the entire BMSM and the reef were selected for the analysis; *i.e.* four observation dates (March '09, May '09, September '09, and January '10). The number of days between a count across the BMSM and on the reef varied between 0 and 12 days. *G*-tests for goodness-of-fit were conducted in order to compare predicted and actual abundances of waders on the reef, assuming no selective use of the reef habitat. A significant total *G*-value means that the data do not fit the expected ratio.

Analysing bird faeces data

First, differences in the frequency of occurrence of benthic taxa among wader species were investigated. Therefore, bird faeces data were transformed into presence/absence data, followed by lumping morphotypes into taxonomic groups. Due to diagnostic features of some morphotypes they could be linked to a certain species. Nonetheless, in

most of the cases it was not possible to assign a morphotype to a species but only to an order, class, or even phylum. For every wader species, the frequency of occurrence for each taxon *i* (FOi%) was calculated: FOi% = (the number of faecal samples of the wader species *s* where taxon *i* is present / the total number of faecal samples of the wader species *s*) * 100. Second, to determine which taxa are preferentially found in faecal samples of particular wader species, the proportion of different taxonomic groups (Ni%) per faecal sample was calculated: Ni% = (Number of items in taxonomic group *i* / total number of items in the faecal sample) * 100. To test whether relative abundances of higher taxonomic groups and the most abundant lower taxonomic groups differed significantly among bird species, a generalized linear model (GLM) was used in the SAS 9.2 software package (Glimmix procedure). Because the response variables are percentage data, the residual error structure was tested against a binomial distribution. When overdispersion became apparent in the model output, the model was rerun, taking the overdispersion into account by adding an overdispersion component (random residual) to the variance function. Accordingly, underestimation of the standard errors was avoided. Because the predictor and the mean response are not linearly related to each other, the relationship was specified by a log link function.

Results

Macrobenthic density, richness, diversity, biomass and production in the *Lanice conchilega* reef

In 2009, 13 806 macroinvertebrates belonging to 61 different taxa were sampled on the reef. Excluding *L. conchilega* itself, the macrobenthic abundance was dominated by the bivalves *Macoma balthica* and *Cerastoderma edule* and the polychaete *Nephtys hombergii*. Taking into account *L. conchilega*, the mean density of macrobenthic species was 6 903 ± 5 339 ind. m^{-2}, *N* = 80; mean species richness was 11.8 ± 4.7 species per square and mean species diversity (H') was 2.1 ± 0.5. At the phylum level, the benthic community within the reef was dominated by annelids (59%), followed by molluscs (38%) and arthropods (1.8%). More than 99.9% of the annelids in the reef belonged to the class Polychaeta. Moreover, this taxon was dominated by *L. conchilega* (69%). The

average *L. conchilega* density was 200 ± 351 ind.m^{-2}, *N* = 150, and a maximum density of 1985 ind.m^{-2} was reached. Within the phylum of Mollusca, the most abundant species were *M. balthica* (55%) and *C. edule* (44%). Crustaceans were the most abundant taxon within the phylum of the arthropods (99.6%). The order of the amphipods (71%) dominated the crustaceans within the reef. Cumaceans, isopods and decapods (crabs) constituted respectively 14.7%, 7.8%, and 6.5% of total abundances. The mean benthic biomass in the reef was 49.7 ± 50.4 g of AFDM.m^{-2}. The annual macrobenthic production in the reef (95% confidence interval) was estimated at 1552.9 (1368.5-1762.3) kJ.m^{-2}.year^{-1} or 70.6 g AFDM.m^{-2}.year^{-1} (60.2-80.1).

Wader density and community composition in the BMSM and on the reef

Across the entire BMSM, 22 wader species were counted, representing a mean density of 1.03 ± 0.58 birds ha^{-1}, *N* = 5. The five most abundant species were: Dunlin (42%), Red Knot (18%), Oystercatcher (13%), Grey Plover (9%) and Curlew (7%). On the reef, 15 wader species were counted, representing a mean density of 51.38 ± 19.11 birds ha^{-1}, *N* = 5. Dunlin (39%) was the most abundant species on the reef followed by Red Knot (20%), Grey Plover (14%), Oystercatcher (13%), Bar-tailed Godwit (8%) and Curlew (5%).

Table 1. Overview of the mean observed and mean expected bird frequencies, and the ratios of these frequencies, for the entire Bay of the Mont Saint-Michel (BMSM) and the L. conchilega *reef; total G-values, and p-values (G-test) of different groups of birds. Observed frequencies in the BMSM and the* L. conchilega *reef were derived from bird counts in March '09, May '09, September '09, and January '10.*

	BMSM			*L. conchilega* reef				
	Obs. Freq. ± SD	Exp. Freq. ± SD	Ratio	Obs. Freq. ± SD	Exp. Freq. ± SD	Ratio	Total *G* value	*p* value
Total waders	23928 ± 15976	29602 ± 17513	0.81	5799 ± 1994	124 ± 74	46.64	140976	<0.0001
5 Species	17651 ± 10761	21830 ± 12882	0.81	4271 ± 2444	92 ± 54	46.58	101915	<0.0002
Dunlin	9853 ± 9478	12080 ± 11337	0.82	2278 ± 1972	51 ± 48	44.89	53565	<0.0003
Oystercatcher	3381± 1963	3916 ± 2502	0.86	551 ± 614	16 ± 11	33.52	12206	<0.0004
Grey Plover	2315 ± 884	3157 ± 1476	0.73	856 ± 79	13 ± 6	64.52	23768	<0.0005
Curlew	1734 ± 1171	1984 ± 1340	0.87	259 ± 274	8 ± 6	31.08	5642	<0.0006
Bar-tailed Godwit	369 ± 400	694 ± 819	0.53	327 ± 425	3 ± 3	112.34	10697	<0.0007

The one-way global ANOSIM test failed to detect a significant difference in the wader community in the two habitats ($p > 0.05$; R = 0.108). Unlike bird composition, bird counts revealed that all wader species together exhibited a high proportion of individuals in the reef in relation to the whole BMSM (Table 1). The frequency of waders in the reef was on average 46.6 times higher than expected assuming a random distribution of waders over the entire BMSM. The same was observed for the five wader species selected for the

faecal analysis. Focussing on each of the 5 wader species separately revealed frequencies ranging from 31.1 times (Curlew) up to 112.3 times (Bar-tailed Godwit) higher than expected. Consequently, the observed numbers of these 5 species in the reef were significantly higher than their predicted numbers (assuming that the total number of waders counted at high tide have a homogeneous distribution in the BMSM at low tide) (for each species, G-test, $p < 0.001$).

Diet of waders feeding on the reef

In general, the frequency of occurrence (FO%) of higher taxonomic groups in the faeces did not differ much among different bird species (Table 2).

Table 2. Frequency of occurrence (FOi%) of higher and the most abundant lower taxonomic groups for all five investigated waders, based on faecal analysis. N = the number of faecal samples investigated. 100% = present in all droppings.

Taxon	*C. alpina* (Dunlin) *N* = 15	*H. ostralegus* (Oystercatcher) *N* = 15	*N. arquata* (Curlew) *N* = 11	*P. squatarola* (Grey Plover) *N* = 15	*L. lapponica* (Bar-tailed Godwit) *N* = 15
Polychaeta	93.3	73.3	72.7	73.3	86.7
Lanice conchilega	40	60	63.6	13.3	33.3
Nereis sp.	6.7	0	9.1	0	0
Eteone longa	0	0	9.1	0	0
Other polychaetes	93.3	73.3	72.7	60	73.3
Crustacea	100	100	100	100	93.3
Crab sp.	73.3	53.3	81.8	80	46.7
Amphipoda sp.	33.3	33.3	45.5	26.7	33.3
Ostracoda sp.	80	73.3	90.9	53.3	40
Isopoda sp.	0	0	9.1	0	6.7
Cumacea sp.	0	0	9.1	0	6.7
Copepoda sp.	0	13.3	0	33.3	20
Other crustaceans	100	100	100	100	93.3
Bivalvia	33.3	13.3	45.5	13.3	26.7
Abra alba	0	6.7	0	6.7	6.7
Aequipecten opercularis	0	0	9.1	0	0
Cerastoderma edule	0	0	36.4	6.7	0
Macoma balthica	20	0	9.1	0	0
Mysella bidentata	0	0	0	0	6.7
Nucula sp.	0	6.7	0	0	0
Scrobicularia plana	0	0	0	0	6.7
Spisula subtruncata	0	0	18.2	0	0
Other bivalves	26.7	0	18.2	0	6.7
Other	100	100	100	100	100

Crustaceans were present in all faecal samples of all birds except for Bar-tailed Godwit (FO% = 93.3%). Both polychaetes and bivalves were present in all bird species but

polychaetes in a much higher percentage of the faeces (ranging from 72.7% in Curlew to 93.3% in Dunlin) than bivalves (ranging from 13.3% in Oystercatcher and Grey Plover to 45.5% in Curlew). *Lanice conchilega* was eaten by all birds though it never exceeded a FO% of 63.6%. Other polychaetes were scarce. Crabs, amphipods and ostracods - the most abundant crustacean groups in the faeces - were encountered in all bird species and reached the highest FO% in Curlew (respectively 81.8%, 45.5% and 90.9%). The three most frequently occurring bivalve species among all bird species were *Abra alba*, *C. edule* and *M. balthica*, although they never exceeded a FO% of 6.7%, 36.4%, and 20%, respectively.

Table 3. Relative abundance (± SD) of all higher and the most abundant lower taxonomic groups in the diet composition of all five investigated wader species, based on faecal analysis. N = the number of faecal samples investigated.

	C. alpina (Dunlin) *N* = 15		*H. ostralegus* (Oystercatcher) *N* = 15		*N. arquata* (Curlew) *N* = 11		*P. squatarola* (Grey Plover) *N* = 15		*L. lapponica* (Bar-tailed Godwit) *N* = 15	
Taxon	%	± SD	%	± SD	%	± SD	%	± SD	%	± SD
Higher taxa										
Polychaeta	17.56	17.57	15.15	24.86	18.17	29.61	5.98	9.59	8.93	18.59
Crustacea	43.31	24.49	26.75	18.16	60.46	35.11	47.26	25.39	18.46	9.65
Bivalvia	0.23	0.40	0.03	0.10	0.07	0.17	0.01	0.03	0.02	0.05
Other	38.90	24.57	58.06	26.07	21.30	26.64	46.75	24.63	72.58	21.24
Polychaeta										
L. conchilega	6.81	12.19	39.79	32.34	20.10	33.57	18.18	40.45	19.68	36.95
Nereis sp.	0.48	1.78	0	0	0.02	0.05	0	0	0	0
E. longa	0	0	0	0	1.01	3.03	0	0	0	0
Other poly.	92.71	12.03	60.21	32.34	78.88	33.32	81.82	40.45	80.32	36.95
Crustacea										
Crab sp.	4.60	5.86	5.12	7.25	32.84	34.00	18.10	18.13	10.06	17.18
Amphipoda sp.	0.81	1.36	1.30	2.34	6.25	19.06	0.46	0.93	5.69	10.23
Ostracoda sp.	9.18	15.06	13.36	17.84	15.81	19.30	8.17	22.37	9.15	13.90
Isopoda sp.	0	0	0	0	0.83	2.74	0	0	0.71	2.67
Cumacea sp.	0	0	0	0	0.001	0.003	0	0	0.71	2.67
Copepoda sp.	0	0	0.92	2.90	0	0	0.41	0.78	1.64	3.56
Other crust.	85.40	19.00	79.30	18.51	44.26	28.28	72.86	25.66	72.04	23.76
Bivalvia										
A. alba	0	0	50.00	70.71	0	0	50.00	70.71	25.00	50.00
A. opercularis	0	0	0	0	2.86	6.39	0	0	0	0
C. edule	0	0	0	0	49.52	47.57	50.00	70.71	0	0
M. balthica	34.50	44.17	0	0	2.86	6.39	0	0	0	0
M. bidentata	0	0	0	0	0	0	0	0	25.00	50.00
Nucula sp.	0	0	50.00	70.71	0	0	0	0	0	0
S. plana	0	0	0	0	0	0	0	0	25.00	50.00
S. subtruncata	0	0	0	0	18.10	26.17	0	0	0	0
Other bivalves	65.50	44.17	0	0	26.67	43.46	0	0	25.00	50.00

Based on the relative abundances of taxonomic groups in each of the 5 bird species a diet composition can be displayed for the 5 bird species examined (Table 3). Relative abundances of all higher taxonomic groups differed significantly ($p < 0.05$) among the 5 wader species (GLM, $p < 0.05$, Table 4).

Table 4. Global p-values (GLM) and adjusted p-values of the pairwise tests (Tukey-Kramer) to check differences in the relative abundances of higher taxonomic groups among the five investigated waders. In case of significant differences (p < 0.05) p-values are in bold. D=Dunlin, O=Oystercatcher, C=Curlew, G=Grey Plover, B=Bar-tailed Godwit.

	Global	D-O	D-C	D-G	D-B	O-C	O-G	O-B	C-G	C-B	G-B
Polychaetes	**0.0254**	0.509	1.000	0.951	0.398	0.197	0.242	1.000	0.894	0.095	0.183
Crustaceans	**< 0.0001**	0.100	0.531	1.000	0.069	**0.0010**	0.124	1.000	0.354	**<0.001**	0.087
Bivalves	**0.0013**	0.307	**0.004**	0.294	0.182	0.999	1.000	0.994	0.998	0.999	0.992
Other	**0.0009**	0.833	0.063	0.894	0.898	**0.002**	1.000	1.000	**0.002**	**0.002**	1.000

Globally, polychaetes differed significantly among species of birds, though no significant pairwise differences were detected. Crustaceans were more frequently eaten by Curlew than by Oystercatcher or Bar-tailed Godwit, while bivalves were eaten more frequently by Dunlin than Curlew. Significant differences in the relative abundances of *L. conchilega* (GLM, $p < 0.0001$) and 'Other polychaetes' (GLM, $p < 0.0001$) were detected among bird species (Table 5).

*Table 5. Global p-values (GLM) and adjusted p-values of the pairwise tests (Tukey-Kramer) to check differences in the relative abundances of lower taxonomic groups (*Lanice conchilega*, Other polychaetes, Crabs and Other crustaceans) among the five investigated wader species. In case of significant differences (p < 0.05) p-values are in bold. D=Dunlin, O=Oystercatcher, C=Curlew, G=Grey Plover, B=Bar-tailed Godwit.*

	Global	D-O	D-C	D-G	D-B	O-C	O-G	O-B	C-G	C-B	G-B
L. conchilega	**< 0.0001**	**0.039**	1.000	0.997	**0.023**	**0.0003**	0.675	0.992	0.997	**0.0003**	0.607
Other poly.	**< 0.0001**	**0.036**	1.000	0.997	**0.022**	**0.0003**	0.672	0.992	0.996	**0.0003**	0.604
Crab	**0.005**	1.000	0.070	0.769	1.000	0.570	0.950	1.000	0.135	0.468	0.950
Other crust.	**< 0.0001**	0.830	**0.0001**	0.704	0.896	0.094	1.000	1.000	**0.0002**	**0.045**	1.000

According to pairwise tests, *L. conchilega* was eaten more frequently by Oystercatcher than by Dunlin and Curlew. Additionally, Bar-tailed Godwit preyed upon *L. conchilega* more frequently than Dunlin, but less than Curlew. Within the group of crustaceans, 'Crabs' differed significantly among bird species (GLM, $p = 0.005$), although no significant pairwise differences were detected. Lastly, no bivalve species showed a significant difference among waders (GLM, $p > 0.05$).

Discussion

Our study showed that the *Lanice conchilega* reef of the BMSM is remarkable because of the wader density which easily exceeds the expected frequency. In the entire BMSM a total of 22 wader species was observed and counted during the study period, while only 15 species were observed in the *L. conchilega* reef. Since the entire BMSM is almost 240 times larger than the reef area, the observed difference in species richness is not surprising keeping in mind the species-area relationship (Connor and McCoy 1979). Consequently, species that were rarely counted at the scale of the entire BMSM are less likely to be observed at the scale of the reef. Despite this difference, both areas were mainly dominated by the same species: Dunlin, Red Knot, Grey Plover, Oystercatcher and Curlew. Eybert *et al.* (2003) already demonstrated that 96% of the wintering shorebird community in the entire BMSM was represented by 7 species: Dunlin, Oystercatcher, Red Knot, Curlew, Grey Plover, Bar-tailed Godwit and Black-tailed Godwit. Overall, the composition of waders can be considered the same in the entire BMSM and the reef.

However, when abundances are included, the composition of waders clearly differed between the two study sites. The observed frequency of total waders on the *Lanice*-reef was on average 5 799 birds, which is approximately 46.6 times higher than expected assuming a uniform distribution of the birds in the BMSM. Petersen and Exo (1999) observed higher than expected bird abundances in *L. conchilega* dominated tidal flats of the Wadden Sea, although densities were only 6 times higher than expected. Similar counting surveys in the Chausey archipelago (France) and preliminary counts in the BMSM already showed the attractiveness of the *L. conchilega* reefs for birds and consequently their potentially important role in the conservation of the avifauna (Godet *et al.* 2008). The results of the current study prove that within a site of international importance for birds, several wader species are able to select preferentially habitats generated by *L. conchilega*.

In general, the attractiveness of *L. conchilega* reefs can be attributed to the good food supply; the high diversity, abundance and biomass of associated macrobenthic invertebrates, as proven by several authors in different study areas (*e.g.* Zühlke *et al.*

1998; Zühlke 2001; Callaway 2006; Rabaut *et al.* 2007a; Van Hoey *et al.* 2008). The situation in the *L. conchilega* reef of the BMSM seems to be alike. In our study, the macrofaunal density is 4.4 times higher than in a study of Trigui (2009), which investigated the general characteristics of the benthic macrofauna of the entire intertidal zone of the BMSM in 2003. Trigui's survey is, with 176 sampled stations, the most extensive benthic survey ever done in the BMSM. Comparison with current research reflects well the fact that the reef does accommodate a more abundant fauna (6 903 ± 5 339 ind.m^{-2}) than the average macrobenthic assemblage in the entire BMSM (1 568 ± 299 ind.m^{-2}). As part of a study of Leloupe *et al.* (2008), biomasses of different trophic groups which make up the BMSM were modelled. The biomass of carnivorous and necrophagous macrobenthic fauna in combination with intertidal filter feeders was transformed according to weight-to-weight conversion factors proposed by Ricciardi and Bourget (1998). Comparing the resulting biomass (2.88 g AFDM. m^{-2}) to the reef biomass (49.69 g AFDM.m^{-2}) revealed a reef biomass which is more than 17 times higher. Therefore, the reef area can be considered a high productivity area in the bay. Nevertheless, given its size, the importance of the whole BMSM for the productivity and functioning of the reef cannot be neglected.

Faecal analysis conducted in this study revealed information on the diet composition of waders in the reef. Polychaetes were represented in all bird species and they occurred in a high percentage of the faecal samples, which can probably be attributed to the dominance of polychaetes in the reef benthos. *Lanice conchilega* was the most abundant species and polychaete in the reef, which was partially reflected in the faecal samples since *L. conchilega* was the only identifiable polychaete species present in all bird species. Nevertheless, based on the relative abundances on the reef it could be expected that *L. conchilega* counted as a larger part of a bird's diet. The fact that the tube-building polychaete is large (up to 30 cm; Hartmann-Schröder 1996), sturdy and buried quite deeply in the sediment (Jones and Jago 1993) can lead to a lower accessibility and possibly explains the lower than expected portion of *L. conchilega* in the diet of waders. Additionally, the fact that the energy content of polychaetes in temperate waters is high (Dauvin and Joncourt 1989), in combination with the high biomass of *L. conchilega* in the reef, might lead to the great contribution of one

individual to the nutritional demands of birds. The bivalves *Macoma balthica* and *Cerastoderma edule* were, next to *L. conchilega*, the most abundant macrobenthic species in the reef. However, the general occurrence and portion of these two species in the diet of the investigated bird species was low. Even in Oystercatchers, which are specialized bivalve feeders (Hulscher 1982), bivalves were underrepresented. A feeding strategy avoiding the uptake of bivalve shell pieces, as described by Hulscher (1982), and hence the low detection success of shell remains in the faeces of Oystercatchers, is the most plausible explanation. Crustaceans, and in particular crabs and ostracods, were the most frequently occurring and abundant taxonomic group in the faeces of all bird species. However, relative abundances of crustaceans in the reef benthos were very low (1.77% of the total abundance), particularly for crabs. The observed ubiquity of crabs (and crustaceans in general) in the faecal samples implies a selective feeding behaviour of waders for this benthic group, which may be due to the fact that the stimuli associated with crustaceans, can have properties which make them particularly perceptible to the avian eye (Goss-Custard 1977).

Based on relative abundances of taxonomic groups, bird species that largely dominate the wader community on the reef exhibited significant differences in their dietary composition. Differences in polychaetes, and especially *L. conchilega*, contributed highly to differences in the wader diets. Differences in diets due to bivalves were rather low and in all probability largely obliterated by an inability to trace back bivalve shells in faeces. For crustaceans, differences in the relative abundance between waders were the result of significant differences in crabs, though no significant pairwise tests were noted. Nevertheless, it can be observed that crabs reached much higher abundances in the faeces of Curlew compared to other waders (Table 3), which is in accordance with studies revealing that crabs are a major food source of Curlew (Goss-Custard and Jones 1976).

Considering the frequency of occurrence of taxonomic groups in the diet, waders foraging within the reef can be seen as opportunistic feeders, while focusing on the relative abundance gives the impression of a more selective feeding strategy. Based on current wader diet analysis, the actual strategy in the reef probably lies somewhere in

between these two extremes. In general, waders feeding on the reef tend to feed on the associated fauna, and especially crustaceans, rather than specifically on *L. conchilega*. This result confirms the investigations of Petersen and Exo (1999), providing evidence that *L. conchilega*, within *L. conchilega* dominated tidal flats, was a less important food source for birds (mainly waders) than the accompanying macrobenthos. However, *L. conchilega* was of overriding importance for a few species, especially for gulls (Petersen and Exo 1999).

As experienced in the current study, investigating bird faeces can give us better insights into the diet of waders feeding in the reef. However, faecal analysis is hampered by a high proportion of unidentified prey items and is unlikely to reveal all prey taken by the predator (Barrett *et al.* 2007). Despite some shortcomings, faecal analysis is a valuable and easily applicable technique for which samples can be obtained all-year round without causing harm to the birds. Moreover, this approach can reveal the presence of prey species which could not be noticed by means of visual observations. Nevertheless, to get a more complete view on a wader's diet, the use of complementary approaches (*e.g.* visual observations) is recommended (Scheiffarth 2001). In this study morphotypes were counted, which cannot be compared with specimens. Since morphotypes are mostly parts of animals and can belong to one or several specimens of the same species or taxonomic group, caution regarding the interpretation of the results is recommended. Faecal samples of the wader species were collected on different dates ranging from the end of April 2010 until the beginning of October 2010. It is known that the diet composition of waders, as well as the nutritive value of some macrobenthic organisms, can change seasonally (*e.g.* Scheiffarth 2001; Braeckman *et al.* 2012). Furthermore, sexual differences in the diet composition have been demonstrated for several birds (*e.g.* Bar-tailed Godwit; Scheiffarth 2001). As neither sexual nor seasonal variations in the diet composition of waders were taken into account in our study, comparing wader diets is restricted. Additionally, future studies should try to compare diet composition of waders both inside and outside the *L. conchilega* reef.

Evaluating the importance of *Lanice conchilega* reefs as trophic resources for waders is not merely fundamental to gain knowledge on the feeding ecology, but is also essential

for predicting the effects of a possible loss of the reef habitat in the future. Overall, the *L. conchilega* reef of the BMSM can be considered an oasis within the tidal flats composed of a similar composition of waders but with much higher bird densities compared to the non-reef areas of the BMSM. This result clearly demonstrates that birds are attracted by the reef. According to faecal analyses, waders in the *L. conchilega* reef tend to feed on the associated fauna, and especially crustaceans, rather than specifically on *L. conchilega* itself. Within the BMSM, which is characterized by low species diversity and low macrofaunal abundances, the *L. conchilega* reef constitutes a rich feeding area. For future studies, the use of complementary techniques to study a wader's diet is recommended, as well as the inclusion of information on the alimentary regime of waders in the BMSM.

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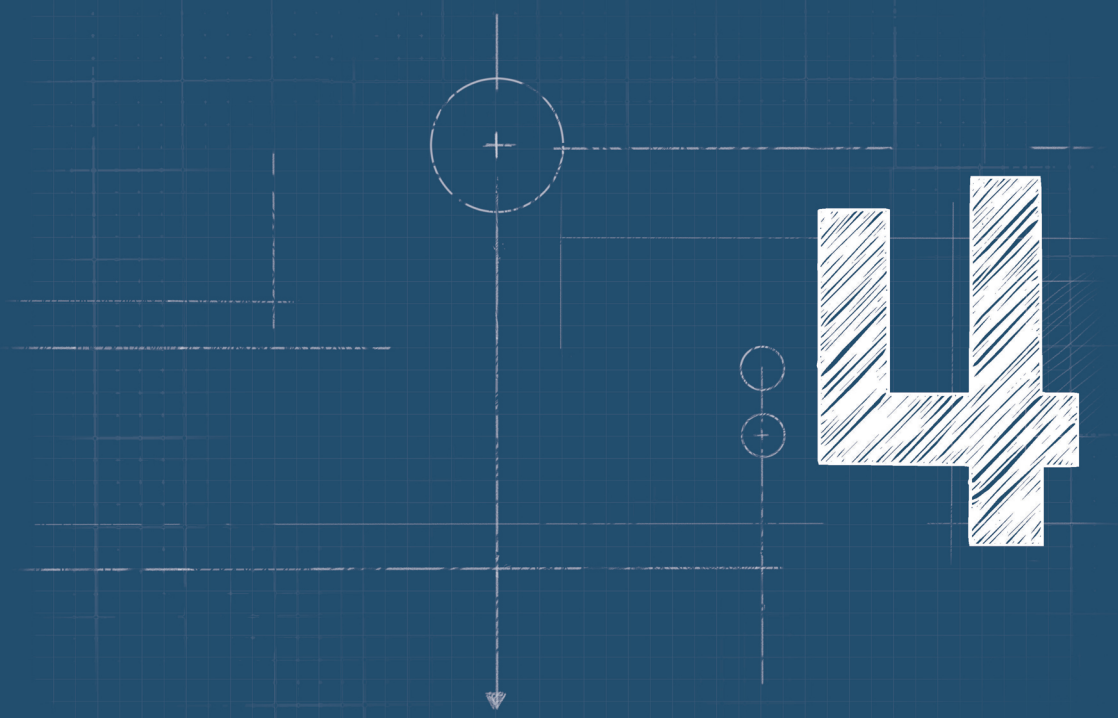

Chapter 4

Predator effects on the feeding and bioirrigation activity of ecosystem-engineered *Lanice conchilega* reefs

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De Smet, B., Braeckman, U., Soetaert, K., Vincx, M., Vanaverbeke, J. (under review) Predator effects on the feeding and bioirrigation activity of ecosystem-engineered *Lanice conchilega* reefs.

Abstract

Ecosystem engineers can considerably affect the community composition, abundance and species richness of their environment. This study investigates the existence of positive or negative feedbacks of species that compose the community in intertidal biogenic reefs constructed by the ecosystem engineer *Lanice conchilega*. This tubeworm creates attractive nursery and feeding grounds for the predatory brown shrimp *Crangon crangon*, while at the same time is preyed upon by *C. crangon*. The effect of the predation pressure exerted by *C. crangon* on the bioirrigation and feeding activity of the tubeworm is up until now unknown and it is hypothesised that these activities are affected by the high densities of *C. crangon* in the reefs. A mesocosm experiment was set-up to investigate the effects of predation pressure on the bioirrigation and feeding activity of *L. conchilega* in the i) absence; ii) restricted presence; and iii) unrestricted presence of *C. crangon*. Bioirrigation was quantified by the decrease of an artificially introduced bromide (Br^-) tracer, while feeding activity was measured from the incorporation of ^{13}C via stable isotope analysis. The bioirrigation rate of the *L. conchilega* reef equalled about 30 L m^{-2} d^{-1} and was not affected by the presence of the predator. The food uptake of the tubeworm was however about three times lower in the unrestricted presence of *C. crangon*, presumably due to the retraction of the worm's body and tentacles in its tube induced by physical contact with the predator. Notwithstanding the impacted food uptake of *L. conchilega*, the tubeworm maintains its functional role in the presence of predators in soft-bottom intertidal areas.

Keywords: bioirrigation, predation, *Lanice conchilega*, *Crangon crangon*, biogenic reef, stable isotopes

Introduction

Most soft-bottom intertidal areas contain limited amounts of biogenic three-dimensional structures, notable exceptions being structures created by ecosystem engineers such as seagrass meadows (*e.g.* Bos *et al.* 2007), macrofaunal feeding tracks and funnels (*e.g.* Volkenborn *et al.* 2007) and biogenic reefs (Holt *et al.* 1998). Ecosystem engineers are organisms that directly or indirectly modulate the availability of resources to other species by reshaping the landscape (Jones *et al.* 1994) and thus change the abiotic context upon which biotic interactions heavily depend (Byers *et al.* 2006). Via these bottom-up and top-down effects, ecosystem engineers considerably affect the community composition, abundance and species richness of their environment, both in positive and negative ways (Jones *et al.* 1997). Some of the impacted species may also affect the population of the ecosystem engineer (Jones *et al.* 1994), especially if the ecosystem engineer is trophically connected to these species (Jones *et al.* 1997; Tribollet and Golubic 2011). This dual role of ecosystem engineers constitutes potentially one of the most important bridges between trophic and non-trophic interactions (Sanders *et al.* 2014).

The increased structural complexity provided by biogenic reefs may offer increased protection from predators (Hixon and Brostoff 1985), but predators can also aggregate within complex habitats (Hixon and Beets 1989), potentially increasing predator-induced mortality. Such negative feedback for instance exists in oyster reef habitats, which provide refugia for the oyster consuming mud crab *Panopeus herbstii* (Grabowski and Powers 2004). Another well-documented example of an ecosystem engineer structurally affecting its environment and at the same time being involved in trophic interactions is the tube-building polychaete worm *Lanice conchilega* (Terebellidae). This species can reach densities of several thousands of individuals per square meter (Van Hoey *et al.* 2006). Above a critical threshold of density and individual tube diameter, surfaces colonised by this species are classified as (biogenic) reef structures (Rabaut *et al.* 2009). Several studies showed that *L. conchilega* reefs have profound structuring impacts on the benthic environment by altering the biogeochemical (Forster and Graf 1995; Braeckman *et al.* 2010) and physical properties of the sediment (*e.g.* Degraer *et al.*

2008; Rabaut *et al.* 2009). The bioirrigation activity of *L. conchilega* (exchanging burrow water with the overlying water, *i.e.* 'piston-pumping') introduces O_2-rich water in the sediment (Forster and Graf 1995). The resulting oxygenation of deeper sediment layers affects the species composition of the smaller interstitial communities (Braeckman *et al.* 2011b) and has important repercussions on benthic respiration, nutrient release and denitrification (Braeckman *et al.* 2010). The increased sediment consolidation and elevation in the presence of the tubeworm (Rabaut *et al.* 2009) provides new habitats and an attractive environment for a large array of species. Moreover, the tube aggregations influence macro-, hyper- and epifaunal densities, species richness and community composition (Zühlke *et al.* 1998; Rabaut *et al.* 2007b; De Smet *et al.* 2015). Lastly, the aggregations may provide refuge from predation, and physical as well as chemical disturbance (Rabaut *et al.* 2007b) and provide attractive feeding grounds for higher trophic levels such as birds (Petersen and Exo 1999; De Smet *et al.* 2013) and flatfish (Rijnsdorp and Vingerhoed 2001; Rabaut *et al.* 2010).

While the importance of *L. conchilega* for higher trophic levels is clear, there is no knowledge on the possible existence of reverse interactions. Both lethal and non-lethal predation of benthic invertebrates by epibenthic species is very common (*e.g.* Berke *et al.* 2009; Maire *et al.* 2010). In order to enhance crypsis (*i.e.* avoiding detection by a predator), benthic infauna can show predator avoidance or predator-mediated behaviour, which can significantly impact the prey's fitness (*e.g.* Meyer and Byers 2005) and ecosystem functions such as bioturbation and organic matter incorporation into the food web (Maire *et al.* 2010).

This study investigates the effects of predation pressure on the bioirrigation and feeding activity of the tubeworm *L. conchilega* in an intertidal reef habitat. The bioirrigation rate quantifies the activity of the *L. conchilega* reef and its impact on the sediment biogeochemistry, while the food uptake will impact the ecosystem engineer's fitness. The brown shrimp *Crangon crangon* was selected as a predator because this species substantially preys upon benthic infauna, including bivalves, amphipods and polychaetes (Pihl and Rosenberg 1984; Norkko 1998) and it is one of the key inhabitants of a *L. conchilega* reef (De Smet *et al.* 2015). We hypothesise that (1) the bioirrigation

activity and (2) the feeding activity of *L. conchilega* are affected by the presence of the predatory shrimp *C. crangon*. The outcome of this study will provide, in combination with previous research on *L. conchilega* reefs, a more global understanding of the interactions involving this particular ecosystem engineer in soft-bottom intertidal areas.

Material & Methods

Study site and sampling

Nine undisturbed *L. conchilega* reef blocks were sampled from the soft-bottom intertidal area of Boulogne-sur-Mer, Nord-Pas-de-Calais (France), along the northeastern part of the English Channel (50°44.01'N-01°35.15'E). The intertidal area of Boulogne was selected because of its well-developed *L. conchilega* reefs, reaching from the intertidal to the subtidal shelf. Sampling took place at the end of the summer of 2014 (the 9th and 10th of September) and during spring low tide. Selected reef blocks had mean tubeworm densities (± SD) of 3243 ± 1094 ind.m^{-2}, resembling the mean *L. conchilega* density of a reef patch in the end of summer (De Smet *et al.* 2015). The reef blocks were sampled with rectangular inox steel frames of 0.12 m^2 (width: 30 cm; length: 40 cm) to a depth of about 18 cm equalling a sample volume of 0.022 m^3. The bottom of the frames was sealed with a removable inox steel plate. Following transportation of the reef blocks to the lab, the frames were replaced by plexiglass aquaria and connected to three continuous recirculation systems (three aquaria per system; each 180 L of aerated, filtered seawater with natural seawater from the sampling location added to it) (Fig. 1). Subsequently, the aquaria were left to acclimatise for 7 days (light regime: 10 h dark/14 h light) before the start of the experiment (Fig. 2). Although the ambient temperature and salinity ranged between 17°C (day 1) and 14.8°C (day 25) and 33.0 (day 1) and 36.5 (day 25) respectively, changes throughout the experiment, were consistent among treatments and replicates. The brown shrimp *Crangon crangon* was caught with a beam trawl (2 m wide, 3 m long, 9 x 9 mm mesh size) at the sandy beach of Mariakerke (Belgium), selected for body size (rostrum-telson length = 37.6 ± 3.1 mm) and left to acclimatize in the lab without food (*i.e.* macrofauna) for at least five days before being added to the aquaria (Fig. 2).

Experimental set-up

The nine aquaria were randomly divided over three replicated (n = 3) treatments: *Control* (*Lanice conchilega* reef block in the absence of *Crangon crangon*), *Shrimp* (*L. conchilega* reef block with *C. crangon* present and unrestricted) and *Net* (*L. conchilega* reef block with *C. crangon* present but physically separated from the reef patch by means of a net). Treatments are hereafter referred to as [C], [S] and [N] respectively.

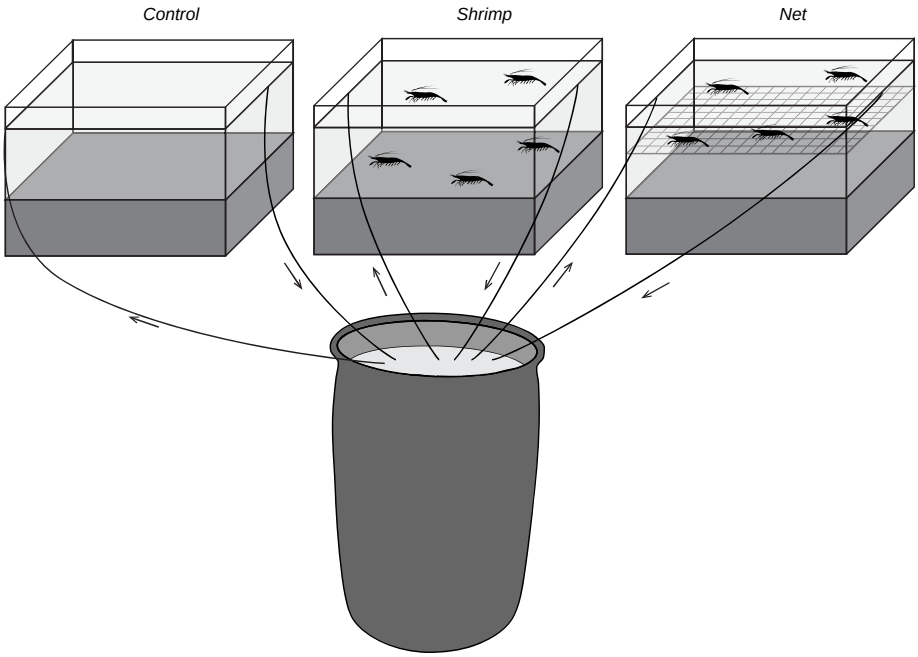

*Figure 1. Experimental set-up. Nine reef blocks were transferred to plexiglass aquaria, connected to three continuous recirculation systems (180 L) and per system randomly divided over three treatments: Control (*Lanice conchilega *reef block in the absence of* Crangon crangon*; left), Shrimp (*L. conchilega *reef block with* C. crangon *present and unrestricted; middle) and Net (*L. conchilega *reef block with* C. crangon *present but physically separated from the reef patch by means of a net; right)*

In order to study the conditions of a *L. conchilega* reef in the presence of a predator, the experiment consisted of (1) a 24 hour bioirrigation activity experiment and (2) a two-weeks feeding activity experiment. At the start of the bioirrigation experiment, all aquaria were disconnected from the recirculation systems, and reconnected after the 24 hour experiment. After a two-day recovery period, the feeding experiment was initiated.

Bioirrigation activity

The bioirrigation activity of *L. conchilega* under different treatments was quantified by the decrease of an artificially introduced bromide (Br^-) tracer in the overlying water column (Glud *et al.* 1996; Rao *et al.* 2012). Br^- is inert in seawater and therefore well-suited for use as a transport rate tracer (Martin and Banta 1992). On day 8 a NaBr stock solution was added to the seawater to a final concentration of about 5-6 mmol.L^{-1}, largely exceeding the *in situ* Br^- concentration (mean ± SE) of 0.84 ± 0.038 mmol.L^{-1} in the used seawater (Fig. 2). To assure a homogeneous mixing of the NaBr-seawater solution, a large volume of the mixture was prepared in an external barrel equipped with a rotating stirrer. Subsequently, a constant volume of 19 L of the NaBr-seawater solution was transferred to each of the aquaria, without disturbing the sediment surface.

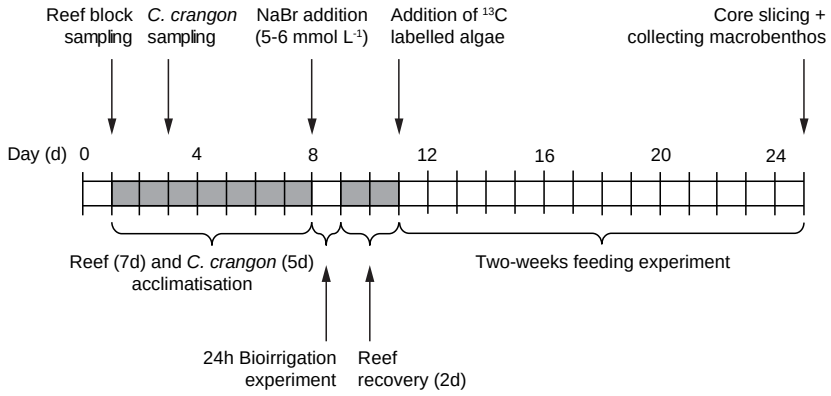

Figure 2. Chronological overview of the 25 days lasting experimental activities including the 24h bioirrigation activity experiment and the two-weeks feeding activity experiment. Grey squares indicate the time periods during which the aquaria were connected to the recirculation systems

A net (3 mm mesh size) was installed in the [N] aquaria and five individuals of *C. crangon* were added to the [S] and [N] aquaria. Each aquarium was aerated by using two diffusive air stones. Prior to the NaBr addition a 2 ml water sample ($T_{reference}$) was taken in order to determine the *in situ* Br^- concentration of seawater. Right after the complete instalment of the aquaria, 2 ml water samples were taken 0, 4, 8, 12 and 24 hours after the addition of the NaBr solution (T_0, T_4, T_8, T_{12} and T_{24} respectively). Samples were kept refrigerated in the dark until further analysis. Bromide anions were separated by means of anion

exchange chromatography (Dionex Ionpac AS14 column) using a 3.5 mM sodium carbonate (Na_2CO_3) and a 1.0 mM sodium bicarbonate ($NaHCO_3$) eluent and detected by using a UV-VIS detector (Dionex Thermo Ultimate-3000) at 210 nm and an electrochemical detector (Dionex ED40). The bromide detection limit was 0.005 mmol.L^{-1}. Relations between the Br^- concentration in the overlying water column and time after addition of NaBr were determined with simple linear regression. The bioirrigation rate *Q* per aquarium ($L.d^{-1}$) (Meysman *et al.* 2007) was estimated as

$$Q = - \frac{V_{OW}}{C_{T_0} - C_{Treference}} \times \frac{dC_{T_0}}{dt}$$

with V_{OW} the volume of overlying water, C_{T0} the Br^- concentration at T_0, $C_{Treference}$ the ambient Br^- concentration of seawater, and dC_{T0}/dt the slope of the linear regressions of Br^- concentration versus time after addition of NaBr. Dividing *Q* by the surface area of the aquarium then provides the bioirrigation rate in $L.m^{-2}.d^{-1}$.

Upon the end of the bioirrigation activity experiment, *C. crangon* and the NaBr-seawater solution were removed from the aquaria, replaced by fresh, filtered seawater and the aquaria were reconnected to the recirculation systems.

Feeding activity

Following a two-day reef recovery period, the feeding activity of *L. conchilega* was investigated in the presence and absence of *C. crangon* by adding ^{13}C labelled algae to the aquaria and measuring the ^{13}C incorporation in the macrofaunal tissue after a two-week period. On day 11, all aquaria were again disconnected from the recirculation systems and 400 mg ^{13}C labelled algal lyophilized cells (99 atom %, Sigma-Aldrich ® 487945) were suspended in 1 L of seawater and homogeneously added to the water column (18 L) of each aquarium by means of a long pipette (Fig. 2). The added algal suspension had a concentration of 1276.69 mg $C.m^{-2}$ (= 1263.92 mg $^{13}C.m^{-2}$).

A net (3 mm mesh size) was installed in the [N] aquaria and five new individuals of *C. crangon* were added to the [S] and [N] aquaria. Each aquarium was aerated by using two diffusive air stones. In order to track the natural, non-enriched $\delta^{13}C$ values of *L. conchilega*, a reference reef block was sampled at the same time and installed in the

same way, but without the addition of labelled algae. The feeding experiment lasted for two weeks because macrobenthic labelling reaches a maximum after about 14 days, followed by an exponential decrease of the ^{13}C label (van Oevelen *et al.* 2006a). At the end of the experiment, three sediment cores (Ø 3.6 cm) were collected from each aquarium and sliced in 1 cm sections to a depth of 5 cm (Fig. 2). Replicate slices were homogenized per aquarium, subsampled for stable isotope analysis and stored frozen at -20°C until further analysis. In order to collect *L. conchilega*, all remaining sediment was sieved through a 1 mm circular mesh size. The *L. conchilega* density per aquarium was determined, individuals were starved in artificial seawater (a mix of Instant Ocean® salt and distilled water; salinity: 33) for 24 h to allow evacuation of their gut contents and stored at -20 °C awaiting further treatment.

Sample preparation and isotope analysis

The sediment samples were lyophilized, grinded with a pestle and mortar, and acidified with 10% HCl to remove inorganic carbonates. About 30 mg of dry sediment was encapsulated in Ag capsules (8 x 5 mm, Elemental Microanalysis UK) and stored dry awaiting stable isotope analysis. Per aquarium, five *L. conchilega* individuals were homogenised, prepared and stored as described in De Smet *et al.* (in press). Samples were analysed for ^{13}C (and organic carbon content) at the Davis Stable Isotope Facility (University of California, USA) using a PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Stable isotope ratios are reported in the standard δ notation as units of parts per thousand (‰) relative to the international V-PDB reference standard:

$$\delta^{13}C = \left[\left(R_{Sample}/R_{Standard}\right)\text{-}1\right] \times 10^3$$

where R is the ratio $^{13}C/^{12}C$, with $R_{Standard} = 0.0112372$.

Incorporation of ^{13}C is reflected as excess (above background) ^{13}C and is expressed as specific uptake ($\Delta\delta^{13}C$, the difference between the $\delta^{13}C$ of the sample after enrichment and the natural $\delta^{13}C$ of the non-enriched sample from the reference aquarium). The bulk uptake of $TO^{13}C$ in the sediment was calculated as the product of excess ^{13}C (E), the organic C content (TOC), the sediment porosity and the sediment dry bulk density

(assumed to be 2.55 g.cm^{-3}). E is the difference between the fraction ^{13}C of the sample (F_{sample}) and the reference ($F_{reference}$), where $F = {}^{13}C/({}^{13}C + {}^{12}C) = R/(R+1)$. The carbon isotope ration (R) was derived from the measured $\delta^{13}C$ values as $R = [(\delta^{13}C/1000)+1] \times R_{Standard}$.

Statistical analysis

Relations between the Br^- concentration in the overlying water column and time after addition of NaBr were tested for every aquarium with simple linear regression. The assumptions for the use of linear regression were tested: normality of the residuals (Shapiro-Wilk test of normality), linearity of the relationship between dependent and independent variables (partial residual plot), residual autocorrelation (Durbin-Watson test) and homoscedasticity (Breusch-Pagan test). One-way Analysis of Variance (ANOVA) was performed to test for differences in the *L. conchilega* density, the bioirrigation rate and the specific uptake of *L. conchilega* between the treatments. In case a significant effect was found, pair-wise tests (Tukey HSD) between the treatments were carried out. Since no significant differences in the *L. conchilega* density were found between treatments (one-way ANOVA, $F_{2,6} = 0.386$, $p = 0.695$), density was not included as a covariable in the analyses. Differences in the $TO^{13}C$ in the sediment remaining at the end of the experiment were tested with a two-way ANOVA, with Treatment and Sediment Depth as two fixed factors. Prior to the ANOVA analyses, the assumptions of normality of the data (Shapiro-Wilk test) and homogeneity of variances (Levene's test) were tested. A significance level of $p < 0.05$ was used in all tests. All statistical analyses were conducted in the open source software R (Version 3.1.2) (R Development Core Team 2015).

Results

Bioirrigation activity

In all aquaria (except for [N1]), a significant linear decrease in the Br^- concentration in the overlying water with time after addition of NaBr was observed (Table 1). The mean bioirrigation rate (± SE) per treatment, derived from the rate of change in the Br^- concentration, was 29.47 ± 1.89 L.m^{-2}.d^{-1} for [C], 29.43 ± 2.08 L.m^{-2}.d^{-1} for [S] and 30.55 ±

0.91 $L.m^{-2}.d^{-1}$ for [N]. The bioirrigation rate was not significantly different between the treatments (one-way ANOVA, $F_{2,6} = 0.1398$, $p = 0.872$).

Table 1. Regression analyses and bioirrigation rates. Simple linear regression of Br^- as a function of the time after addition of NaBr for the three treatments in triplicate: [C] = Control, [S] = Shrimp, and [N] = Net. The bioirrigation rate is based on the slope (α) and the initial (T_0) and ambient (T_t) Br- concentrations of the overlying water. Significant regressions ($p < 0.05$) are in bold.

Treatment	Linear regression analyses				Bioirrigation rate
	α	β	adj. R^2	*p* (slope)	($L\ m^{-2}\ d^{-1}$)
[C1]	5.5675	-0.0420	0.9561	**0.0026**	32.9412
[C2]	5.5304	-0.0335	0.8481	**0.0169**	26.4492
[C3]	5.6268	-0.0377	0.8314	**0.0199**	29.0117
[S1]	5.6416	-0.0362	0.8913	**0.0101**	27.8631
[S2]	5.4284	-0.0433	0.7293	**0.0415**	33.5454
[S3]	5.5810	-0.0345	0.7739	**0.0313**	26.8868
[N1]	5.3661	-0.0407	0.6373	0.0660	31.9376
[N2]	5.7945	-0.0405	0.9827	**0.0006**	30.8960
[N3]	6.0045	-0.0379	0.8341	**0.0194**	28.8243

Feeding activity

An immediate increase in the feeding activity of *L. conchilega*, as seen from the appearance of tentacles outside the sandy fringe, was observed for all aquaria upon addition of the ^{13}C labelled algae to the overlying water. The mean specific uptake ($\Delta\delta^{13}C$) (±SE) of *L. conchilega* after 14 days was lowest in the unrestricted presence of *C. crangon* [S] (837 ± 276‰) and much higher in the absence [C] (2414 ± 218‰) and the restricted presence of *C. crangon* [N] (2396 ± 1023‰) (Fig. 3). Differences in the mean $\Delta\delta^{13}C$ of *L. conchilega* were significant between the treatments (one-way ANOVA, $F_{2,6} = 6.311$, $p = 0.033$), and pair-wise tests showed a significantly higher specific uptake in [C] compared to [S] (TukeyHSD; $p = 0.048$).

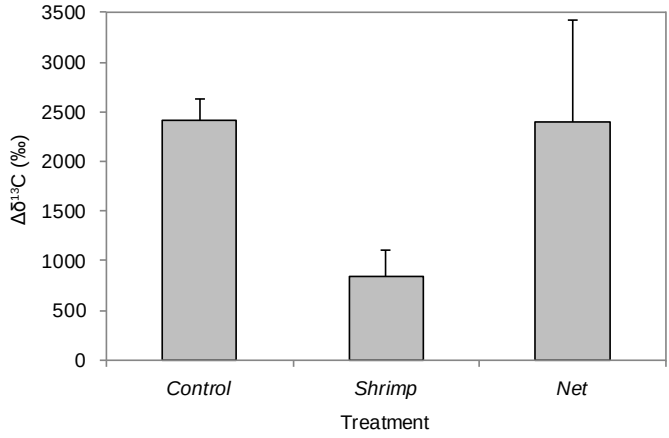

Figure 3. The incorporation of ^{13}C in Lanice conchilega*, expressed as mean specific uptake ($\Delta\delta^{13}C$) (±SE), for the three treatments: Control, Shrimp, and Net*

About 11% of the $TO^{13}C$ initially added to the experiment remained in the upper cm of the sediment after 14 days and only a minor fraction (2%) of the initially added $TO^{13}C$ was transported down to a depth of 5 cm (Fig. 4). Neither the concentration, nor the vertical distribution of $TO^{13}C$ differed significantly for the different treatments (two-way ANOVA, $F_{2,12} = 0.1759$, $p = 0.841$).

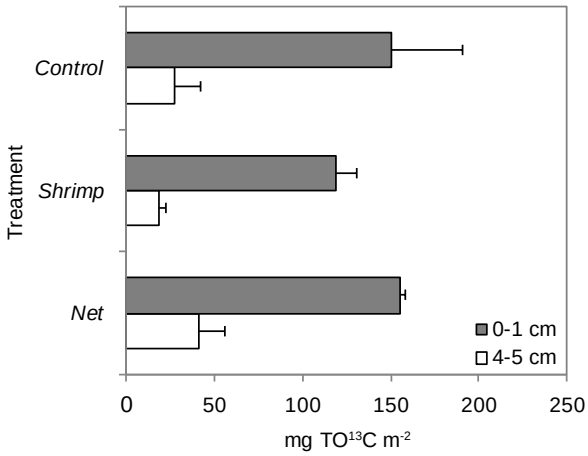

Figure 4. Mean (±SE) $TO^{13}C$ (in mg m^{-2}) remaining in the sediment at the end of the feeding activity experiment for the three treatments (Control, Shrimp, and Net) and at two depths (0-1 cm and 4-5cm)

Discussion

Bioirrigation activity

The steep decrease in the Br^- concentration in the overlying water revealed the pronounced bioirrigation activity of the *Lanice conchilega* reef, which is in line with the existing knowledge (Forster and Graf 1995; Braeckman *et al.* 2011a). However, so far only microcosm and single-species approaches were used to demonstrate the bioirrigation capacity of *L. conchilega*. The *L. conchilega* reef approach used in this study more faithfully reflects the natural situation. Moreover, while previous studies measured the vertical penetration of oxygen as a proxy for bioirrigation (Forster and Graf 1995; Braeckman *et al.* 2011a), this is to our knowledge the first study that makes use of an inert tracer to directly quantify the bioirrigation activity of *L. conchilega*. The use of a tracer is advantageous because it allows the exchange of a solute independent of other chemical or biological processes such as sediment respiration (Martin and Banta 1992).

The bioirrigation rate of the reef blocks equalled about 30 $L.m^{-2}.d^{-1}$, which is higher than the species-specific bioirrigation rates of *L. conchilega* (12 $L.m^{-2}.d^{-1}$) in subtidal silty fine sand in the North Sea (Forster and Graf 1995), but much lower than the bioirrigation rates of natural populations of the polychaetes *Arenicola marina* (120-160 $L.m^{-2}.d^{-1}$), *Nereis virens* (60-180 $L.m^{-2}.d^{-1}$) and *Nereis diversicolor* (580-720 $L.m^{-2}.d^{-1}$ for non-suspension feeding and 2600-2900 $L.m^{-2}.d^{-1}$ for suspension feeding *N. diversicolor*) (Kristensen 2001). These differences reflect the fact that bioirrigation rates are species-specific and depend on the physical mechanism used (Kristensen 2001; Shull *et al.* 2009; Kristensen *et al.* 2014). Studies on natural populations of burrowing shrimp report bioirrigation rates ranging from 3.20 $L.m^{-2}.d^{-1}$ in *Callianassa subterranea* (Forster and Graf 1995) up to 429 $L.m^{-2}.d^{-1}$ in *Trypaea australiensis* (Webb and Eyre 2004). However, the latter study makes use of a caesium chloride (CsCl) tracer, which is adsorbed to sediment particles, and hence causes an overestimation of transport rates (Webb and Eyre 2004).

The bioirrigation activity did not appear to be impacted by the presence of the predatory brown shrimp *Crangon crangon*. The tubeworm uses piston-pumping to ventilate its tube, *i.e.* water is drawn into the tube and pushed out whenever the worm

moves within its tube (Forster and Graf 1995). As *L. conchilega* does not necessarily have to eject its body and tentacles in the water-column, and risk an encounter with a predator, this activity can be maintained in the presence of the predator. Whilst we did not measure the effect of predator-prey interactions on the biogeochemistry of the sediment, it can be assumed that the unchanged bioirrigation rate in the *L. conchilega* reef in the presence of a predator does not affect the biogeochemistry of the sediment.

Feeding activity

Lanice conchilega is a surface deposit feeder but has the ability to switch to suspension feeding in case the amount of food on the bottom is limited (Buhr 1976; Fauchald and Jumars 1979). Suspension feeding is especially advantageous to avoid competition in areas of high densities (*i.e.* several thousand ind.m^{-2}; Buhr (1977)). Upon addition of the labelled algae in the experiment, a quick and intense suspension feeding response of the polychaete was observed for all aquaria regardless the treatment. Once the algae had settled on to the sediment surface, the initial suspension feeding activity markedly decreased (pers. obs.). Stable isotope analysis showed that the feeding activity of *L. conchilega* was negatively affected by the unrestricted presence of the predator, but feeding was not significantly depressed when the predator was physically separated from the worm by means of a net. In contrast to its bioirrigation performance, the polychaete has to eject its tentacles beyond the fringe of its tube in order to feed, and therefore becomes vulnerable to predation. The lowered food uptake of *L. conchilega* in the direct presence of *C. crangon* can therefore be attributed to a predator avoidance behaviour, consisting of the withdrawal of its body and tentacles in its tube; reducing the feeding activity to sub-optimal levels. Reduced feeding activity was only recently shown to affect ecosystem processes such as bioturbation (Maire *et al.* 2010). Predator avoidance behaviour is expected to occur mainly during night, when *C. crangon* feeds most actively (Pihl and Rosenberg 1984). This might explain the observed burrowing behaviour of *C. crangon* and the occasional feeding activity of *L. conchilega* in all aquaria during daytime (pers. obs.). In the restricted presence of the predator, the food uptake of the polychaete remains high, implying that the impediment of *L. conchilega*'s feeding behaviour is not triggered by chemical signals induced by *C. crangon*. Maire *et al.* (2010)

suggest that it is not advantageous to detect the presence of distant predators when predators are continuously present. The uninterrupted predation pressure exerted by the high abundances of *C. crangon* in reefs constructed by *L. conchilega* probably results in behavioural changes induced by physical contact with the predator, as is assumed to be the case for the bivalve *Macoma balthica* (Maire *et al.* 2010).

The TO^{13}C remaining in the sediment did not vary significantly between treatments, indicating an unaltered uptake of ^{13}C labelled algae in the absence or presence of the predator. Since *L. conchilega* was shown to have a decreased uptake in the unrestricted presence of *C. crangon*, the algal material might be taken up by other macrofaunal species in the reef blocks, which consisted mainly of the polychaetes *Eumida sanguinea*, *Phyllodoce mucosa* and *Nephtys cirrosa*, and the amphipod *Urothoe poseidonis*. Nevertheless, *L. conchilega* makes up ±82% of the macrofaunal biomass in the reef (De Smet *et al*. unpublished), and is hence the most important species in the reef. Therefore, macrofaunal species associated to *L. conchilega* were not further taken into account in this study.

Feedback effects

A negative feedback from the predator to the ecosystem engineer can be expected when the engineering activity increases the abundance of predators (Sanders *et al.* 2014). *L. conchilega* attracts large quantities of predatory shrimp by creating a heterogeneous environment, functioning as an important nursery and feeding area (De Smet *et al.* 2015). The current study shows that a reciprocal effect of *C. crangon* on the feeding activity of the engineering tubeworm takes place, while the bioirrigation activity remains unaltered in the presence of the predator. Therefore, the assumed predator avoidance and/or predator-mediated behaviour in the presence of *C. crangon* does not affect the allogenic ecosystem engineering activity of the tubeworm and the resulting oxygenation of deeper sediment layers. In contrast, the food uptake is three times higher in the absence of *C. crangon*. In natural reefs, *C. crangon* is continuously present, inducing a sub-optimal feeding by the tubeworm, without impeding the bioirrigation activity. The unchanged ecosystem engineering activity is important in maintaining the bottom-up effects by which the ecosystem engineer influences the

community composition, abundances and species richness of the associated fauna (Jones *et al.* 1997). Our findings are not entirely consistent with the study of Maire *et al.* (2010), who showed a reduced feeding activity and a strong effect on the bioturbation intensity of *M. balthica* in the presence of *C. crangon*. Hence, the effect of the predator-prey interaction depends on the type of engineering that is being affected (bioturbation vs. bioirrigation) and the associated life history traits of the ecosystem engineer. The potential of a species to engineer its environment depends on its mobility in the sediment and its ability for sediment reworking, two traits that affect the biological sediment mixing and therefore the biogeochemistry of the sediment (Solan *et al.* 2004). The extent to which these traits are affected by the predator determines the effect of the predator-prey interaction on the sediment biogeochemistry. The sediment reworking capacity of *M. balthica*, and tellenid bivalves in general, is tightly linked to their feeding activity (Maire *et al.* 2006; Maire *et al.* 2007), which changed drastically in the presence of a predator (Maire *et al.* 2010). In the presence of *C. crangon*, *M. balthica* increases its mobility and sediment reworking capacity, resulting in an increased thickness of the bioturbated sediment layer and non-local transport of sediment particles at depth (Maire *et al.* 2010). Contrastingly, *L. conchilega* affects the biogeochemistry of the sediment by means of its bioirrigation activity (Forster and Graf 1995; Braeckman *et al.* 2010). The presence of *C. crangon* does however not affect the bioirrigation capacity of *L. conchilega* since both the mobility and the sediment reworking capacity of the tubeworm remain unchanged. The tube-dwelling life-mode of *L. conchilega* and the ability to retreat in its tube in the presence of a predator can explain the tubeworm's capacity to continue its movement in its tube, and thus its bioirrigation activity, despite its interrupted feeding.

Conclusion

Ecosystem engineers create attractive environments for other species, which in turn may affect ecosystem engineering activities (Wright and Jones 2006). *L. conchilega* creates an attractive nursery and feeding area for *C. crangon*. In turn, the predatory shrimp exerts a trophic pressure on the tubeworm which affects its feeding activity, but not its bioirrigation activity. The unaltered bioirrigation activity enables *L. conchilega* to have a

functional role in soft-bottom intertidal areas, even under high predatory pressure. In order to generalise this result to other ecosystem engineers, the type of engineering activity, whether or not linked to feeding activity, and the associated life-history traits of the engineer, have to be taken into account.

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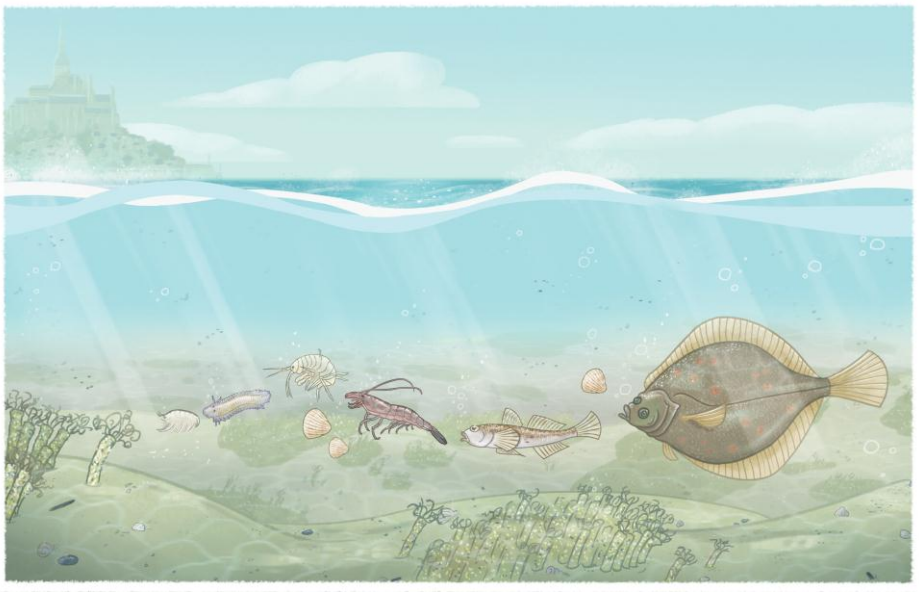
5

Chapter 5

Integrating ecosystem engineering and food web ecology: testing the effect of biogenic reefs on the food web of a soft-bottom intertidal area



Modified from the publication:

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Abstract

The potential of ecosystem engineers to modify the structure and dynamics of food webs has recently been hypothesised from a conceptual point of view. Empirical data on the integration of ecosystem engineers and food webs is however largely lacking. This paper investigates the hypothesised link based on a field sampling approach of intertidal biogenic reefs created by the ecosystem engineer *Lanice conchilega* (Polychaeta, Terebellidae). The reef structures are known to have a considerable impact on the physical and biogeochemical characteristics of their environment and subsequently on the abundance and biomass of primary food sources and the macrofaunal (*i.e.* the macro-, hyper-, and epibenthos) community. Therefore, we hypothesise that *L. conchilega* reefs affect the structure, stability and isotopic niche of the consumer assemblage of a soft-bottom intertidal food web. Primary food sources and the bentho-pelagic consumer assemblage of a *L. conchilega* reef and control area were sampled on two soft-bottom intertidal areas along the French coast and analysed for their stable isotopes. Despite the structural impacts of the ecosystem engineer on the associated macrofaunal community, the presence of *L. conchilega* reefs only has a minor effect on the food web structure of soft-bottom intertidal areas. The isotopic niche width of the consumer communities of reef and control areas are highly similar, implying that most consumer taxa do not shift their diet when feeding in a *L. conchilega* reef. Nevertheless, an indirect engineering effect of the tubeworm on a minor fraction of the consumer taxa was observed. Besides, species packing and hence trophic redundancy were not affected, pointing to an unaltered stability of the food web in the presence of *L. conchilega*.

Keywords: ecosystem engineer, food web structure, biogenic reef, community metrics, *Lanice conchilega*, intertidal area, stable isotopes

Introduction

Ecosystem engineers (species that contribute to the creation, modification or maintenance of the physical environment, which therefore have a crucial effect on other species (Jones *et al.* 1994)) and food webs are both well documented. The incorporation of non-trophic interactions in traditional food web studies is however only recently increasing (*e.g.* Olff *et al.* 2009; Kéfi *et al.* 2012), and up till now, the significance of the common and often influential process of ecosystem engineering on food web structure and dynamics remains largely unknown (Sanders *et al.* 2014). To get a more general understanding of interaction webs in nature, the integration of ecosystem engineering and food webs cannot be longer avoided (Sanders *et al.* 2014). Sanders *et al.* (2014) recently presented a conceptual framework to integrate the largely independent research areas of ecosystem engineering and food webs. By structurally changing the abiotic environment, engineers can impact the structure of food webs either via node modulation (effect on the number of species that are present and their densities) or via link modulation (effect on the number and strength of trophic and non-trophic interactions among species) (Fig.1). The former also includes a subsequent change in links from the nodes to the rest of the food web (Sanders *et al.* 2014). Node and link modulation can operate on three non-exclusive engineering pathways; they can change the abiotic conditions (*e.g.* temperature and pH), the consumable abiotic conditions (*e.g.* nutrient leaching) and the non-trophic resources (*e.g.* competitor-free space). Via these pathways, the engineer might facilitate the addition of new producer species or alter the producer biomass and as such affect higher trophic levels (Sanders *et al.* 2014). The engineering pathways are believed to influence a food web at four possible levels: one trophic level, a food web compartment, a sub-set of species at different trophic levels or all species in the food web (Sanders *et al.* 2014). Moreover, if the engineer is trophically coupled to the food web (as a producer, consumer or decomposer), the net effect of the engineer on the food web will depend on a combination of engineering effects, trophic effects and positive or negative feedbacks to the engineer (Sanders *et al.* 2014).

Despite the growing interest in the capacity of ecosystem engineers to modify the structure and dynamics of food webs, most studies dealing with this issue have a

theoretical nature and empirical evidence is largely lacking (Arditi *et al.* 2005; Goudard and Loreau 2008; Sanders *et al.* 2014). So far, only a few recent studies have been looking at the link combining both research fields in the marine realm (*e.g.* Rigolet *et al.* 2014b; Rigolet *et al.* 2015). More evidence on how ecosystem engineers might affect food web structure and stability can be best provided by making use of an ecosystem engineer combining both autogenic (changing the environment via their own physical structures) and allogenic (changing the environment by transforming living or non-living materials from one physical state to another) engineering capacities (Jones *et al.* 1994). The terebellid polychaete *Lanice conchilega* is a prime example of an organism proven to be both an autogenic and allogenic ecosystem engineer (Godet *et al.* 2008; Rabaut *et al.* 2009). On the one hand, *L. conchilega* alters the biogeochemical properties of the environment by its bioirrigating activities, while on the other hand it creates biotic surface structures referred to as biogenic reefs (Carey 1987; Rabaut *et al.* 2009; Callaway *et al.* 2010; Godet *et al.* 2011), hence providing new habitats. In the presence of this engineering species, positive biodiversity and/or abundance and biomass effects have been reported on different size and/or ecological groups, ranging from primary producers (sediment particulate organic matter (SPOM) and microphytobenthos (MPB); De Smet *et al.* 2015; De Smet et al. unpublished) to smaller meiofauna (*e.g.* Zühlke *et al.* 1998; Braeckman *et al.* 2011b) and associated macrobenthos (*e.g.* Zühlke 2001) and hyperbenthos (De Smet *et al.* 2015) up to (juvenile) (flat)fish and waders (*e.g.* Godet *et al.* 2009; De Smet *et al.* 2013; Rabaut *et al.* 2013).

While the structural and functional role of biogenic reefs has been well investigated, their impact on the food web structure has only been poorly considered. Following the conceptual framework of Sanders *et al.* (2014), the habitats created by *L. conchilega* are expected to impact the overall food web structure via node modulation, since the tubeworm alters the primary producers' abundance and biomass, and subsequently affect the nodes (*i.e.* species abundance and biomass) of the macrofaunal food web (De Smet *et al.* 2015; De Smet et al. unpublished) (Fig. 1; based on Sanders *et al.* 2014). Therefore, we investigated the potential effect of biogenic *Lanice conchilega* reefs on the structure of the macroscopic soft-bottom intertidal food web.

We combined a classical approach (functional groups) and a more integrative approach based on stable isotope analysis to study the food web structure. In ecological studies, the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ stable isotope ratios are the most frequently used to infer primary food sources, trophic linkages and trophic position (Layman *et al.* 2012).

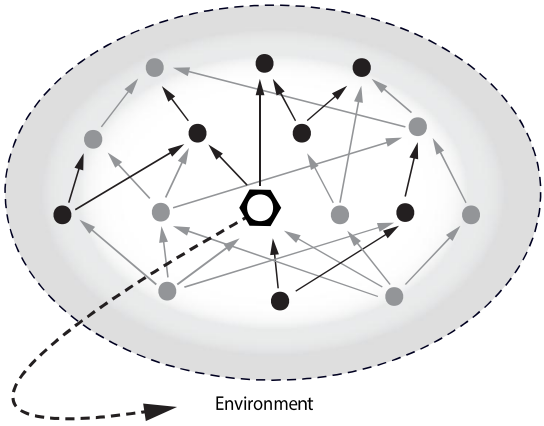

Figure 1. Schematic representation of the expected impact of the ecosystem engineer Lanice conchilega *on the structure of a soft-bottom intertidal food web. The engineer (⬡), which is trophically coupled to the food web, affects the physical and biogeochemical characteristics of the environment (dotted arrow) and hence the base (primary producers) and higher trophic levels (macrofauna) of the food web. Consequently, the changes in the environment are expected to impact the overall structure of the food web (greyscale gradient). Nodes represent the primary producers and macrofaunal food web compartments, while arrows represent the trophic interactions, before (black) and after (grey) alteration by the engineer (based on Sanders et al. 2014)*

Generally, the quantitative information on both resource and habitat use provided by stable isotope analysis is utilised to define isotopic niche: an area (in δ-space) with isotopic values (δ-values) as coordinates (Newsome *et al.* 2007). Although tightly correlated, the isotopic niche should not be confused with an animal's trophic niche (Layman *et al.* 2012); a hypervolume in n-dimensional space with environmental variables as axes (Hutchinson 1957). Layman *et al.* (2007) introduced a number of metrics that make use of stable isotope data to describe trophic structure ranging from individuals to entire communities and which can be used to compare trophic structures across systems or time periods. By implementing Bayesian statistics, Jackson *et al.* (2011) provided improved estimates of the community metrics allowing for statistical comparison of the isotopic niches of communities, both in space and time.

This study uses stable isotopes to investigate whether changes in the species composition in the presence of *L. conchilega* also cause changes in the structure of the food web. Moreover, to our knowledge, this is the first study that investigates the effect of an ecosystem engineer on the food web structure by using Bayesian Layman metrics. More specifically, we tested if the altered abundance and biomass of primary producers and macrofauna in the presence of *L. conchilega* affect the structure, stability and the isotopic niche of the consumer assemblage of a soft-bottom intertidal food web. Rather than focussing on one single ecosystem component, the entire bentho-pelagic consumer assemblage associated with the intertidal *L. conchilega* reef (De Smet *et al.* 2015), was taken into account. To exceed the local scale, two different intertidal areas, representing different environmental settings along the French coast, were investigated.

Material & Methods

Study area and sampling design

Two soft-bottom intertidal areas located along the French side of the English Channel were sampled for primary food sources and consumer species. The Bay of the Mont Saint-Michel (BMSM) is a large-scale intertidal sand flat located in the Normand-Breton Gulf (48°39.70' N-01°37.41' W; France); while Boulogne-sur-Mer (further referred to as Boulogne) is a soft-bottom intertidal along the northern part of the English Channel (50°44.01'N-01°35.15'E; Northern France). The locations were selected based on the presence of a well-established intertidal *L. conchilega* reef.

At each location, the main primary food sources in the area (SPOM and MPB) and consumer species were sampled within a *L. conchilega* reef-dominated area and a (control) area in the absence of any bioengineering species. The bathymetric level between the reefs and their respective control areas was similar and the sampling areas were at least 300 m apart from each other. To include temporal variability in isotopic values, sampling took place in spring 2012 (from 7th till 13th of March in the BMSM and from 22nd till 25th of March in Boulogne) and was repeated in autumn 2012 (from 17th till 21st of September in BMSM and from 15th till 18th of October in Boulogne). Sampling was

conducted in cooperation with the National Museum of National History (MNHN, Paris, France) and permitted by the '*Affaires Maritimes*'.

Sampling of sources and consumers

Sediment particulate organic matter (SPOM) was collected by sampling the upper cm of the sediment during low tide. Upon return at the lab, artificial seawater was added to the sediment and following sonication and sieving (38 μm), the supernatant was filtered onto precombusted (450°C for 2h) and pre-weighed Whatman GF/F filters (25 mm), temporarily stored frozen at -20°C and subsequently at -80°C until processing. Fresh microphytobenthos (*i.e.* benthic diatoms; MPB_{diatom}) was collected by transferring the upper cm of the sediment to plastic boxes, covering the sediment with 100 x 150 mm Whatman lens cleaning tissue and cover slides, and putting the sediment under controlled light conditions enabling diatom migration. After about 2 days, diatoms were scraped off the cover slides, transferred to flacons with milli-Q water and centrifuged at 3000 rpm for 3 min. The diatom pellets were transferred to Sn capsules (30 mm Ø, Elemental Microanalysis UK), dried at 60°C and subsequently pinch closed and stored in Multi-well Microtitre plates under dry atmospheric conditions awaiting further analysis.

Macrobenthic invertebrates were collected with an inox corer (Ø 12 cm, 40 cm deep), sieved through a 1 mm circular mesh size and stored in a bucket with seawater. Upon return in the lab, animals were sorted, identified to the lowest possible taxonomic level, starved in seawater (24h) to allow evacuation of their gut contents and stored at -20°C before further treatment. In order to study the epi- and hyperbenthic communities, the lower water column (up to 40 cm) covering the sampling areas was sampled during daytime ebbing tide. Fish, shrimp and other epibenthic organisms were sampled with a beam trawl (2m long, 3m wide, 9x9 mm mesh size) equipped with a tickler-chain in the ground rope. Similarly, smaller animals living in the water layer close to the seabed (hyperbenthos; Mees and Jones 1997) were collected with a hyperbenthic sledge consisting of a metal frame (100x40cm) and equipped with two identical nets: a lower and an upper net (3 m long, 20 cm high at the mouth, 1x1 mm mesh size). The beam trawl and the hyperbenthic sledge were towed, either by a speedboat (Sillinger) in the BMSM or by foot in Boulogne, at a speed of 1 knot in the surf zone and parallel to the

coastline for 100 m. Catches were sorted, identified to the lowest possible taxonomic level, starved in seawater (24h) to allow evacuation of their gut contents (only in case of smaller invertebrates), and stored at -20°C before further treatment. For each combination of location (BMSM *vs.* Boulogne), sampling area (reef *vs.* control area) and period (spring *vs.* autumn), 3 macrobenthic cores, 1 hyperbenthic catch and 1 epibenthic catch were collected.

Sample preparation and stable isotope analysis

GF/F filters of SPOM and all collected consumer species were prepared for ^{13}C and ^{15}N stable isotope analysis. Frozen filters were thawed, dried overnight at 60°C and subsequently acidified by exposing them to HCl fumes (37%) in a dessicator to remove inorganic carbonates (Lorrain *et al.* 2003). Filters were re-dried overnight at 60°C before being enclosed in Sn capsules (30 mm Ø, Elemental Microanalysis UK). In case of smaller invertebrates, such as polychaetes, amphipods and mysids, entire individuals were selected, whereas for fish and larger invertebrates, such as bivalves, crab and the brown shrimp *Crangon crangon*, only muscular tissue (dorsal fin, foot, cheliped and tail muscle tissue respectively) was prepared for analysis. Entire specimens and the dissected tissue samples were rinsed thoroughly with milli-Q water to avoid contamination and subsequently dried overnight at 60°C. Dried samples were grinded with a pestle and mortar, homogenized, weighed and encapsulated. The selection of the capsule depends on the need for acidification to remove carbonate traces (Jacob *et al.* 2005), which was tested in advance. Invertebrates with calcareous structures such as shrimp, isopods and juvenile crab were transferred to Ag capsules (8 × 5 mm, Elemental Microanalysis UK) and acidified by adding dilute (10%) HCl drop-by-drop, until no more release of CO_2 was observed (Jacob *et al.* 2005). Following acidification, samples were rinsed with milli-Q water, dried, pinch closed and stored in Multi-well Microtitre plates under dry atmospheric conditions until analysis. Carbonate-free tissue samples on the other hand, were encapsulated in Sn capsules (8 × 5 mm, Elemental Microanalysis UK), closed and immediately stored dry awaiting further analysis.

In total, 399 samples (both filters and animal tissue) were analysed at the UC Davis Stable Isotope Facility (University of California, USA) using a PDZ Europa ANCA-GSL

elemental analyser, interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Stable isotope ratios are reported in the standard δ notation as units of parts per thousand (‰) relative to the international reference standards:

$$\delta X = \left[\left(R_{Sample}/R_{Standard}\right) - 1\right] \times 10^3$$

where X is ^{13}C or ^{15}N and R is the corresponding ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Reference standards used were Vienna-Pee Dee Belemnite limestone (V-PDB) for carbon and atmospheric N_2 for nitrogen. At least three replicates of SPOM and MPB_{diatom} were analysed for each combination of location, sampling area and period. In case of consumers, we strived to analyse at least three replicates per species (every replicate equalled one single individual), but for several taxa less replicates were available.

Data analysis

This study incorporated two different sampling locations (BMSM and Boulogne) along the French coastal area. Analysis of the stable isotope data was performed for each of the locations separately since they are characterized by different environmental settings.

Primary food sources

Differences in the $\delta^{13}C$ and $\delta^{15}N$ isotope values of the primary food sources (SPOM and MPB_{diatom}) between levels of the fixed factors sampling area (reef *versus* control) and period (spring *versus* autumn) were tested by a two-way ANOVA (Analysis of Variance). Significant interaction effects ($p < 0.05$) were further investigated by means of a TukeyHSD test. Prior to ANOVA, the assumptions of normality and homogeneity of variances were tested on untransformed data with Shapiro-Wilk tests and Levene tests respectively.

Consumers

The carbon and nitrogen isotope composition of consumer taxa co-occurring in both the reef and control areas were compared by plotting them in $\delta^{13}C$ and $\delta^{15}N$ biplots. Separate biplots were created for the locations and within biplots a distinction was made between periods. Taxa were assumed to have resembling $\delta^{13}C$ and $\delta^{15}N$ values in

the reef and control areas if they fell within the 95% confidence interval (CI) encompassing the 1:1 correlation between reef and control isotope values.

To provide a detailed description of the entire food web structure, we used a classical approach and a more integrative approach. The classical approach includes the clustering of taxa in trophic groups; the integrative approach consists of the estimation of community-wide metrics based on Bayesian statistics. Classification of consumers in groups of individuals with similar food uptake ($\delta^{13}C$) and trophic level ($\delta^{15}N$) was achieved by means of agglomerative hierarchical cluster analyses with group-average linking (Clarke and Gorely 2006). Clustering was performed for each of the combinations of location, sampling area and period separately and applied on a Euclidean distance resemblance matrix of normalised $\delta^{15}N$ and $\delta^{13}C$ isotope values of individual consumers. The clusters were examined for significant differences by similarity profile (SIMPROF) permutation tests (Clarke and Gorely 2006). Cluster analysis and SIMPROF test were performed using PRIMERv6 (Clarke and Gorely 2006). Furthermore, based on literature (*e.g.* Fauchald and Jumars 1979) and the World Register of Marine Species (WoRMS; http://www.marinespecies.org/) consumers were classified into 8 functional groups: fish, predators, omnivore/predator/scavengers, omnivores, deposit feeders/facultative suspension feeders, suspension feeders, deposit feeders and herbivores.

The structure and niche widths of the food webs were investigated by calculating 6 descriptive community-wide metrics based on stable isotope data. The metrics were originally proposed by Layman *et al.* (2007) and reformulated in a Bayesian framework by Jackson *et al.* (2011). Trophic diversity within the community is reflected by the total extent of spacing within $\delta^{13}C$ - $\delta^{15}N$ biplot space and measured by the first four metrics: *$\delta^{15}N$ range* (NR; representation of the vertical food web structure), *$\delta^{13}C$ range* (CR; representation of diversity at the base of the food web), *total area* of the convex hull encompassing the data (TA; representation of the niche space occupied) and *mean distance to centroid* (CD; representation of the average trophic diversity within the food web). The extent of trophic redundancy (the relative position of taxa to each other within niche space) is measured by metrics five and six: *mean nearest neighbour distance* (MNND) and *standard deviation of the nearest neighbour distance* (SDNND). These metrics

were calculated based on standard ellipses (Batschelet 1981) and Bayesian methods resulting in improved estimates, including their uncertainty (Jackson *et al.* 2011). However, because the TA is highly sensitive to sample size and hence impedes comparison between communities with unequal sample sizes, Bayesian standard ellipse area (SEA_B) was used. Standard ellipses are not sensitive to sample size because they generally contain about 40% of the data. Nevertheless, for small sample sizes ($n < 30$) the tendency towards underestimating the SEA remains. Therefore, a small sample-size corrected standard ellipse (SEA_c), insensitive to sample size (Jackson *et al.* 2011), was calculated.

All univariate analyses were run in R (Version 3.1.2). The calculation of the Bayesian Layman's metrics and standard ellipse areas for the different communities was done using SIBER (Stable Isotope Bayesian Ellipses in R; Jackson *et al.* 2011).

Results

Primary Food Sources

In the BMSM, MPB_{diatom} (ranging from -14.08 ± 4.07‰ to -6.84 ± 0.63‰) showed a more enriched $\delta^{13}C$ value than SPOM (ranging from -22.66 ± 0.66‰ to -20.79 ± 0.39‰) for all sampling areas and periods (Fig. 2). The $\delta^{13}C$ value of both SPOM and MPB_{diatom} was affected by the factor period: $\delta^{13}C$ of SPOM was lower in spring than in autumn, while $\delta^{13}C$ of MPB_{diatom} was higher in spring than in autumn (Table 1). $\delta^{15}N$ values in the BMSM ranged from 3.72 ± 0.38‰ (SPOM in the reef area during spring) to 7.21 ± 1.60‰ (MPB_{diatom} in the control area during autumn) (Fig. 2). No differences in $\delta^{15}N$ of MPB_{diatom} could be detected, while the $\delta^{15}N$ value for SPOM differed among the sampling area x period interaction (Table 1). However, only in autumn the $\delta^{15}N$ value of SPOM was significantly higher in the reef area compared to the control area (Table 1).

In Boulogne, MPB_{diatom} (ranging from -19.58 ± 1.18‰ to -12.67 ± 3.97‰) showed a more enriched $\delta^{13}C$ value than SPOM (ranging from -24.60 ± 0.70‰ to -21.21 ± 0.10‰) for all sampling areas and periods (Fig. 3). The $\delta^{13}C$ value of both SPOM and MPB_{diatom} was affected by the interaction of sampling area x period (Table 1). Pair-wise tests showed that the $\delta^{13}C$ value of SPOM in the reef area was significantly higher than in the control

area but only in autumn (Table 1). No significant pair-wise differences could be detected for MPB_{diatom}. $\delta^{15}N$ values in Boulogne range from 0.91 ± 0.99‰ (MPB_{diatom} in the control area during spring) to 4.66 ± 0.77‰ (SPOM in the reef area during spring) (Fig. 3). Nor for SPOM, neither for MPB_{diatom} differences in $\delta^{15}N$ for the factors sampling area and period could be detected (Table 1).

Table 1. Two-way Analysis of Variance (ANOVA) and pair-wise Tukey HSD test results for the carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope values of the primary food sources (SPOM and MPB_{diatom}). Sampling area (reef vs. control) and period (spring vs. autumn) were fixed factors. Analyses were performed on untransformed stable isotope data and run separately for the Bay of the Mont Saint-Michel (BMSM) and Boulogne-Sur-Mer (Boulogne). Pair-wise Tukey HSD test results were shown for significant sampling area x period interactions. In case of significant differences (p < 0.05) p values are in bold.

	Main test												**Pair-wise test**	
	Sampling area				Period				Sampling area x Period				Spring	Autumn
Source	SS	df	F-value	*p* value	SS	df	F-value	*p* value	SS	df	F-value	*p* value	*p* adj	*p* adj
BMSM														
$\delta^{13}C$ SPOM	0.14	1	0.6839	0.4322	3.04	1	15.0586	**0.0047**	0.15	1	0.7305	0.4176	—	—
$\delta^{15}N$ SPOM	1.38	1	12.564	**0.0076**	1.69	1	15.318	**0.0045**	1.73	1	15.749	**0.0041**	0.2418	**0.0310**
$\delta^{13}C$ MPB_{diatom}	14.55	1	3.3218	0.0956	32.30	1	7.3747	**0.0201**	0.00	1	0.0001	0.9943	—	—
$\delta^{15}N$ MPB_{diatom}	3.58	1	2.9043	0.1164	0.60	1	0.4891	0.4989	1.76	1	1.4264	0.2575	—	—
Boulogne														
$\delta^{13}C$ SPOM	2.76	1	17.715	**0.0030**	17.20	1	110.39	**<0.0001**	2.65	1	17.009	**0.0033**	0.4186	**0.0126**
$\delta^{15}N$ SPOM	0.001	1	0.002	0.9655	0.30	1	0.568	0.4726	0.26	1	0.4827	0.5069	—	—
$\delta^{13}C$ MPB_{diatom}	30.20	1	4.2622	0.0659	71.55	1	10.097	**0.0099**	55.33	1	7.8073	**0.0190**	0.2924	0.2289
$\delta^{15}N$ MPB_{diatom}	2.43	1	3.828	0.0821	1.06	1	1.6756	0.2277	0.03	1	0.0517	0.8253	—	—

Consumers

In total, 346 organisms belonging to 71 taxa were collected and analysed for their stable isotope values. 188 organisms (46 taxa) inhabiting the BMSM were analysed, of which 97 organisms (36 taxa) were collected in the reef area and 91 organisms (38 taxa) in the control area; while for Boulogne 158 organisms (42 taxa) were analysed, of which 82 organisms (34 taxa) were collected in the reef area and 76 organisms (30 taxa) in the control area. The majority of organisms in the BMSM were crustaceans (52.1%) and fish (18.6%), as was the case for Boulogne (40.5% en 39.9% resp.) (Table 2 and 3). Both for the BMSM (Fig. 2) and Boulogne (Fig. 3), the most depleted $\delta^{13}C$ values were found in the reef area during spring (BMSM: *Lekanesphaera monodi* = -23.45‰; Boulogne: *Pleuronectes platessa* = -24.99‰) and the most enriched $\delta^{13}C$ values in the control area during autumn (BMSM: *Crangon crangon* = -11.75‰; Boulogne: *Liocarcinus sp.* = -

12.71‰). Regarding $\delta^{15}N$, the most depleted (*Lekanesphaera monodi* = 2.62‰) and most enriched (*Pomatoschistus sp.* = 15.93‰) values for the BMSM were found in the reef area during spring, while for Boulogne the most depleted (*Crangon crangon* juvenile = 6.46‰) and most enriched (*Syngnathus rostellatus* = 16.68‰) values were found in the control area during autumn. In general, most taxa fell within a 1:1 correlation of isotope values between a reef and control area, *i.e.* their $\delta^{13}C$ and $\delta^{15}N$ values in the reef areas resembled those of the control areas (Fig. 4).

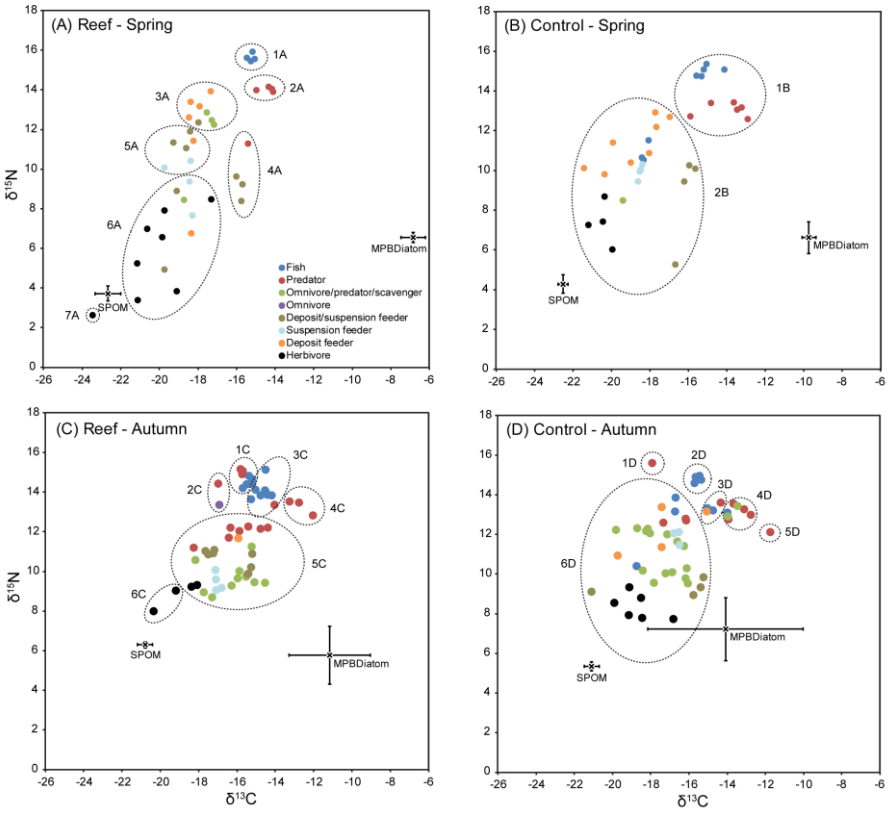

Figure 2. Biplot of $\delta^{13}C$ and $\delta^{15}N$ isotope values (‰) of the primary food sources (mean ± SD) and of individuals of consumer taxa of the soft bottom intertidal area of the Bay of the Mont Saint-Michel (BMSM) for different combinations of sampling area (reef vs. control) and period (spring vs. autumn). A = reef – spring; B = control – spring; C = reef – autumn; D = control – autumn. Colours represent the 8 different functional groups (fish, predator, omnivore/predator/scavenger, omnivore, deposit/facultative suspension feeder, suspension feeder, deposit feeder, herbivore). Dashed ellipses represent the trophic groups delineated based on agglomerative hierarchical cluster analyses and similarity profile (SIMPROF) permutation tests. The mean $\delta^{13}C$ and $\delta^{15}N$ values (±SD) of the clusters, as well as their taxonomic composition are listed in Appendix 1

However, isotope values for some taxa were enriched (on the right side of the 95% CI) or depleted (on the left side of the 95% CI) in the reef compared to the control area. In the BMSM during autumn, enriched $\delta^{13}C$ values in the reef were found for *Idotea linearis*, *Diogenes pugilator* and *Loligo vulgaris*, while no taxa showed depleted $\delta^{13}C$ values.

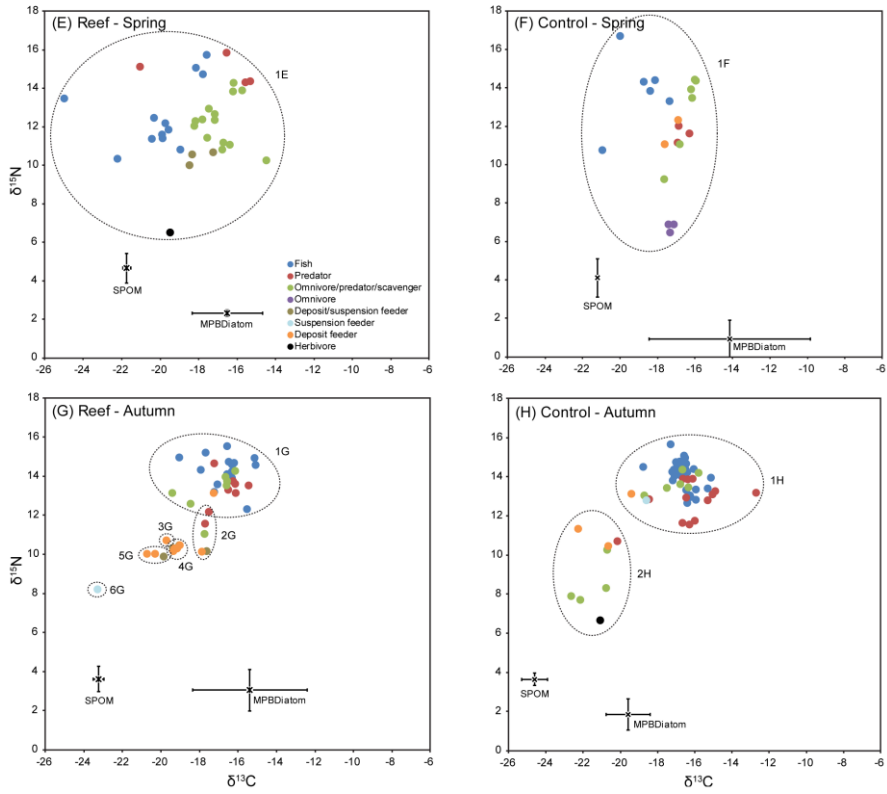

Figure 3. Biplot of $\delta^{13}C$ and $\delta^{15}N$ isotope values (‰) of the primary food sources (mean ± SD) and of individuals of consumer taxa of the soft bottom intertidal area of Boulogne-sur-Mer for different combinations of sampling area (reef vs. control) and period (spring vs. autumn). E = reef – spring; F = control – spring; G = reef – autumn; H = control – autumn. Colours represent the 8 different functional groups (fish, predator, omnivore/predator/scavenger, omnivore, deposit/facultative suspension feeder, suspension feeder, deposit feeder, herbivore). Dashed ellipses represent the trophic groups delineated based on agglomerative hierarchical cluster analyses and similarity profile (SIMPROF) permutation tests. The mean $\delta^{13}C$ and $\delta^{15}N$ values (±SD) of the clusters, as well as their taxonomic composition are listed in Appendix 2

In contrast, in spring, *Crangon crangon* showed a depleted $\delta^{13}C$ value in the reef, while no taxa showed enriched $\delta^{13}C$ values. In Boulogne during autumn, *Nephtys cirrosa* showed a depleted $\delta^{13}C$ value in the reef, while during spring *Syngnathus rostellatus*

showed an enriched $\delta^{13}C$ value in the reef. Regarding $\delta^{15}N$, in spring some taxa exhibited enriched values in the reef (*Schistomysis kervillei* and *Crangon crangon* in the BMSM; *Mesopodopsis slabberi* in Boulogne) while *Macoma balthica* showed a depleted value in the reef. In autumn, no taxa with enriched or depleted $\delta^{15}N$ values in the reef were found (Fig. 4).

Table 2. Stable carbon and nitrogen isotope values (‰, mean ± SD if appropriate) of the primary food sources and consumer taxa of the soft bottom intertidal area of the Bay of the Mont Saint-Michel (BMSM) for different combinations of sampling area (reef vs. control) and period (spring vs. autumn). n = the number of replicates. FG = the functional group in which the consumer taxon was classified (1 = fish, 2 = predator, 3 = omnivore/predator/scavenger, 4 = omnivore, 5 = deposit/facultative suspension feeder, 6 = suspension feeder, 7 = deposit feeder, 8 = herbivore).

		Reef						Control					
		Spring			Autumn			Spring			Autumn		
Taxon	FG	$\delta^{13}C$ (SD)	$\delta^{15}N$ (SD)	n	$\delta^{13}C$ (SD)	$\delta^{15}N$ (SD)	n	$\delta^{13}C$ (SD)	$\delta^{15}N$ (SD)	n	$\delta^{13}C$ (SD)	$\delta^{15}N$ (SD)	n
Osteichthyes													
Dicentrarchus labrax	1	-	-	-	-14.51	15.13	1	-14.12	15.07	1	-	-	-
Pleuronectes platessa	1	-	-	-	-	-	-	-	-	-	-15.51	13.24	3
Platichthys flesus	1	-	-	-	-	-	-	-	-	-	-13.99	13.10	1
Pomatoschistus sp.	1	-15.23 (0.17)	15.64 (0.21)	4	-15.32 (0.14)	14.58 (0.19)	4	-15.29 (0.22)	14.97 (0.28)	4	-15.77 (0.53)	14.62 (0.45)	5
Atherina presbyter	1	-	-	-	-15.71	14.20	1	-	-	-	-	-	-
Solea solea	1	-	-	-	-14.67 (0.38)	13.92 (0.17)	7	-	-	-	-	-	-
Syngnathus rostellatus	1	-	-	-	-	-	-	-18.27 (0.18)	10.89 (0.54)	3	-18.73	10.39	1
Crustacea													
Crangon crangon	2	-14.38 (0.39)	14.02 (0.11)	4	-13.02 (0.84)	13.30 (0.33)	4	-13.30 (0.31)	13.05 (0.35)	4	-13.14 (1.13)	13.08 (0.70)	4
Liocarcinus sp.	2	-	-	-	-	-	-	-	-	-	-13.53 (0.58)	13.02 (0.35)	2
Carcinus maenas	3	-	-	-	-18.17	10.57	1	-	-	-	-	-	-
Athanas nitescens	3	-18.71	8.45	1	-	-	-	-	-	-	-	-	-
Palaemon serratus	4	-	-	-	-16.91	13.36	1	-	-	-	-	-	-
Portumnus latipes	3	-	-	-	-	-	-	-	-	-	-13.70 (0.36)	13.17 (0.38)	2
Processa sp.	3	-	-	-	-	-	-	-	-	-	-19.13 (0.99)	10.80 (0.87)	2
Eualus cranchii	3	-	-	-	-	-	-	-	-	-	-18.58 (0.63)	12.23 (0.12)	4
Philocheras trispinosus	3	-	-	-	-	-	-	-	-	-	-16.19 (0.11)	10.16 (0.18)	2
Diogenes pugilator	3	-	-	-	-15.59 (0.93)	9.45 (0.19)	3	-	-	-	-16.74 (0.82)	9.74 (0.27)	4
Corophium volutator	5	-	-	-	-	-	-	-	-	-	-15.76	8.94	1
Corophium sp.	5	-	-	-	-	-	-	-16.68	5.26	1	-	-	-
Gammarus sp.	8	-19.71 (1.48)	7.03 (1.25)	5	-18.99 (1.02)	8.90 (0.61)	4	-20.49 (0.52)	7.33 (1.09)	4	-19.17 (0.58)	8.66 (0.58)	4
Abludomelita obtusata	5	-19.10	8.89	1	-	-	-	-	-	-	-	-	-
Melita sp.	5	-19.72	4.93	1	-	-	-	-	-	-	-	-	-
Bathyporeia elegans	7	-18.32	6.76	1	-	-	-	-	-	-	-	-	-
Schistomysis kervillei	7	-18.05 (0.45)	12.91 (0.96)	5	-	-	-	-17.59 (0.44)	12.15 (0.91)	4	-	-	-
Schistomysis spiritus	6	-18.78 (0.81)	9.38 (1.50)	3	-	-	-	-	-	-	-16.61 (0.16)	11.89 (0.37)	3
Gastrosaccus spinifer	7	-	-	-	-	-	-	-19.76 (0.69)	10.52 (0.80)	3	-18.57 (1.64)	11.16 (0.31)	2
Mesopodopsis slabberi	3	-	-	-	-	-	-	-	-	-	-17.54 (0.61)	11.94 (0.27)	3
Lekanesphaera levii	8	-21.22 (2.18)	3.28 (0.62)	3	-	-	-	-	-	-	-17.63 (1.15)	7.77 (0.04)	2
Idotea linearis	3	-	-	-	-15.49 (0.39)	9.74 (0.28)	3	-19.39	8.48	1	-16.61	9.54	1
Idotea balthica	3	-	-	-	-17.52 (0.31)	8.81 (0.17)	2	-	-	-	-	-	-
Diastylis sp.	5	-	-	-	-	-	-	-	-	-	-21.11	9.11	1
Mollusca													
Loligo vulgaris	2	-	-	-	-16.05 (0.62)	14.89 (0.32)	4	-	-	-	-17.92	15.62	1
Buccinum undatum	2	-	-	-	-14.85 (0.51)	12.21 (0.05)	3	-14.81	13.38	1	-	-	-
Cerastoderma edule	6	-18.42	9.37	1	-17.03 (0.15)	9.47 (0.47)	4	-18.50 (0.10)	9.92 (0.36)	4	-	-	-
Macoma balthica	5	-15.81 (0.16)	9.08 (0.62)	3	-15.32 (0.11)	10.21 (0.48)	4	-15.94 (0.29)	9.91 (0.42)	3	-15.32 (0.11)	9.60 (0.36)	2
Annelida													
Lanice conchilega	5	-18.55 (0.54)	11.67 (0.57)	4	-17.40 (0.22)	10.97 (0.10)	4	-	-	-	-	-	-
Arenicola marina	7	-	-	-	-15.94	11.67	1	-	-	-	-	-	-
Nephtys cirrosa	2	-	-	-	-16.21 (0.29)	11.99 (0.26)	3	-15.88	12.71	1	-16.56 (0.69)	12.71 (0.10)	3
Nephtys sp.	2	-15.40	11.30	1	-	-	-	-	-	-	-	-	-
Nereis sp.	3	-	-	-	-15.24	11.24	1	-	-	-	-	-	-
Polynoinae sp.	3	-17.31 (0.20)	12.52 (0.31)	3	-	-	-	-	-	-	-	-	-
Scoloplos armiger	7	-	-	-	-	-	-	-	-	-	-16.25 (1.68)	13.28 (0.16)	2
Oligochaeta sp.	7	-	-	-	-	-	-	-21.42	10.09	1	-	-	-
Cnidaria													
Rhizostoma pulmo	2	-	-	-	-18.25	11.19	1	-	-	-	-	-	-
Primary food sources													
SPOM		-22.66 (0.66)	3.72 (0.38)	3	-20.79 (0.39)	6.30 (0.16)	3	-22.52 (0.26)	4.28 (0.47)	3	-21.10 (0.40)	5.34 (0.23)	3
MPB_{diatom}		-6.84 (0.63)	6.56 (0.25)	4	-11.17 (2.12)	5.77 (1.46)	4	-9.74 (0.36)	6.62 (0.80)	4	-14.08 (4.07)	7.21 (1.60)	3

Table 3. Stable carbon and nitrogen isotope values (‰, mean ± SD if appropriate) of the primary food sources and consumer taxa of the soft bottom intertidal area of Boulogne-sur-Mer for different combinations of sampling area (reef vs. control) and period (spring vs. autumn). n = the number of replicates. FG = the functional group in which the consumer taxon was classified (1 = fish, 2 = predator, 3 = omnivore/predator/scavenger, 4 = omnivore, 5 = deposit/facultative suspension feeder, 6 = suspension feeder, 7 = deposit feeder, 8 = herbivore).

		Reef							Control					
		Spring			Autumn				Spring			Autumn		
Taxon	FG	$\delta^{13}C$ (SD)	$\delta^{15}N$ (SD)	n	$\delta^{13}C$ (SD)	$\delta^{15}N$ (SD)	n		$\delta^{13}C$ (SD)	$\delta^{15}N$ (SD)	n	$\delta^{13}C$ (SD)	$\delta^{15}N$ (SD)	n
Osteichthyes														
Dicentrarchus labrax	1	-	-	-	-16.73 (1.34)	14.69 (0.37)	4		-	-	-	-16.54 (0.05)	15.04 (0.08)	2
Pleuronectes platessa	1	-24.99	13.47	1	-16.29 (1.09)	12.94 (0.90)	2		-	-	-	-15.96 (0.54)	13.24 (0.41)	7
Pleuronectidae sp.	1	-	-	-	-	-	-		-20.95	10.74	1	-	-	-
Pleuronectidae juv.	1	-20.48 (1.34)	11.24 (0.91)	4	-	-	-		-	-	-	-	-	-
Pleuronectidae larvae	1	-19.77 (0.15)	11.76 (0.34)	4	-	-	-		-	-	-	-	-	-
Pomatoschistus microps	1	-	-	-	-16.77 (1.63)	14.47 (0.55)	4		-	-	-	-16.90 (0.13)	14.38 (0.32)	3
Pomatoschistus sp.	1	-17.96 (0.24)	14.89 (0.24)	2	-16.38 (0.13)	14.31 (0.39)	4		-	-	-	-16.76 (0.29)	14.45 (0.25)	10
Scopthalmus rhombus	1	-	-	-	-17.24	13.18	1		-	-	-	-16.58 (0.67)	13.85 (0.74)	4
Echiichthys vipera	1	-	-	-	-16.58	15.53	1		-	-	-	-17.31	15.67	1
Syngnathus rostellatus	1	-17.59	15.74 -	1	-	-	-		-20.00	16.68	1	-	-	-
Sprattus sprattus	1	-	-	-	-16.48	14.74	1		-	-	-	-	-	-
Ammodytidae sp.	1	-	-	-	-	-	-		-18.16 (0.59)	13.96 (0.51)	4	-18.77	14.50	1
Echinodermata														
Psammechinus miliaris	2	-	-	-	-	-	-		-16.93	11.14	1	-	-	-
Crustacea														
Crangon crangon	2	-17.13 (2.66)	14.92 (0.73)	4	-15.99 (0.37)	13.50 (0.27)	4		-	-	-	-16.39 (0.22)	13.65 (0.48)	4
Crangon crangon juv.	4	-	-	-	-	-	-		-17.29 (0.15)	6.74 (0.24)	3	-	-	-
Liocarcinus sp.	2	-	-	-	-	-	-		-	-	-	-15.35 (2.90)	13.10 (0.21)	3
Liocarcinus sp. juv.	2	-	-	-	-	-	-		-	-	-	-20.15	10.71	1
Carcinus maenas	3	-16.05 (0.27)	14.00 (0.26)	3	-16.49 (0.23)	13.88 (0.31)	4		-16.08 (0.12)	14.04 (0.44)	4	-16.62 (0.62)	13.81 (0.43)	5
Carcinus maenas juv.	3	-14.48	10.26	1	-	-	-		-	-	-	-	-	-
Praunus flexuosus	6	-	-	-	-	-	-		-	-	-	-18.59	12.80	1
Eualus cranchii	3	-	-	-	-	-	-		-	-	-	-18.72	13.04	1
Gammarus sp.	8	-19.48	6.50	1	-	-	-		-	-	-	-21.07	6.66	1
Urothoe poseidonis	7	-	-	-	-19.66 (1.25)	10.23 (0.32)	4		-	-	-	-	-	-
Urothoe sp. juv.	7	-	-	-	-19.21 (0.15)	10.31 (0.13)	4		-	-	-	-	-	-
Nototropis swammerdamei	3	-	-	-	-	-	-		-	-	-	-21.85 (0.97)	7.96 (0.31)	3
Schistomysis kervillei	7	-	-	-	-	-	-		-16.91	12.31	1	-	-	-
Mesopodopsis slabberi	3	-16.86 (0.49)	11.12 (0.25)	4	-	-	-		-17.23 (0.58)	10.15 (1.30)	2	-	-	-
Gastrosaccus spinifer	7	-	-	-	-	-	-		-17.62	11.07	1	-20.78 (1.43)	11.63 (1.36)	3
Eurydice pulchra	3	-	-	-	-	-	-		-	-	-	-20.70	10.26	1
Mollusca														
Venerupis sp.	6	-	-	-	-23.29	8.20	1		-	-	-	-	-	-
Buccinum undatum	2	-	-	-	-	-	-		-	-	-	-15.32	12.80	1
Annelida														
Lanice conchilega	5	-18.02 (0.68)	10.41 (0.35)	3	-18.99 (0.96)	10.21 (0.25)	4		-	-	-	-	-	-
Arenicola marina	7	-	-	-	-17.27	13.14	1		-	-	-	-	-	-
Nephtys cirrosa	2	-	-	-	-17.61 (0.12)	11.88 (0.43)	2		-16.59 (0.40)	11.82 (0.28)	2	-16.33 (0.34)	11.64 (0.10)	3
Glycera alba	2	-	-	-	-16.89 (0.51)	13.98 (0.95)	2		-	-	-	-	-	-
Pholoe minuta	3	-17.81	12.39	1	-	-	-		-	-	-	-	-	-
Phyllodoce mucosa	3	-18.23	12.05	1	-18.10 (0.52)	11.82 (1.09)	2		-	-	-	-	-	-
Polynoinae sp.	3	-17.49 (0.48)	12.56 (0.29)	4	-	-	-		-	-	-	-	-	-
Lumbrineris sp.	3	-	-	-	-19.42	13.14	1		-	-	-	-	-	-
Notomastus sp.	7	-	-	-	-19.27	10.26	1		-	-	-	-	-	-
Cnidaria														
Actiniaria sp.	2	-	-	-	-	-	-		-	-	-	-15.04	13.11	1
Primary food sources														
SPOM		-21.74 (0.22)	4.66 (0.77)	3	-23.24 (0.28)	3.62 (0.66)	3		-21.21 (0.10)	4.10 (1.01)	3	-24.60 (0.70)	3.64 (0.32)	3
MPB_{diatom}		-16.51 (1.83)	2.32 (0.17)	4	-15.38 (2.97)	3.05 (1.06)	4		-12.67 (3.97)	0.91 (0.99)	3	-19.58 (1.18)	1.85 (0.80)	3

Classical approach towards the effect of *L. conchilega* on the food web structure

Following the cluster analysis (and SIMPROF test) of consumers based on their isotope values, the number of trophic groups in a reef area was either equal to (BMSM - autumn and Boulogne – spring) or at least thrice as high (BMSM – spring and Boulogne - autumn) as the number of clusters in a control area. The number of functional groups

between reef and control areas was equal, but in the cases where the trophic groups in a reef area were thrice as high, functional groups were distributed over more trophic groups (Fig. 2 and 3; see Appendices 1 and 2 for taxa included in each trophic group).

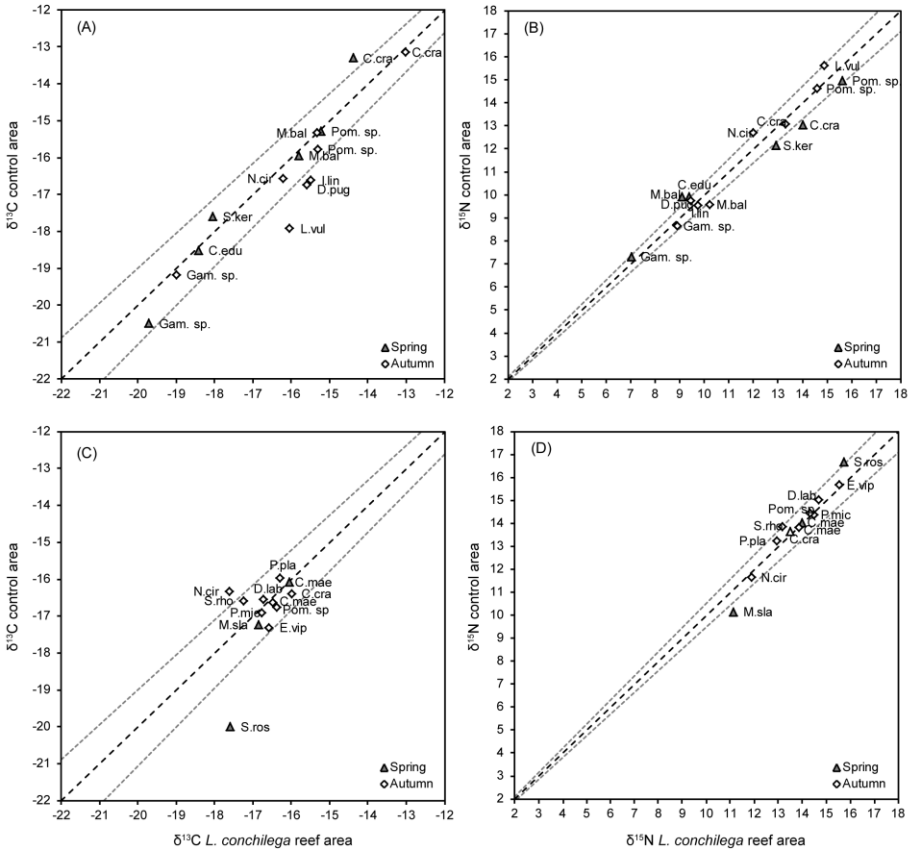

Figure 4. Comparison of consumer isotope values between the Lanice conchilega *reef areas and the control areas. Separate plots for $\delta^{13}C$ and $\delta^{15}N$ isotope values per location are displayed: $\delta^{13}C$ values in the BMSM (A) and Boulogne (C); $\delta^{15}N$ values in the BMSM (B) and Boulogne (D). The central dashed line represents a 1:1 correlation between isotope values in the reef vs. control areas. A 95% confidence interval is represented by the outer dashed lines. Consumer taxa within the 95% confidence interval are not significantly different between reef and control areas. Only consumer taxa which were collected both in the reef and control areas were taken into account. Species abbreviations: C. mae* = Carcinus maenas*; C. edu* = Cerastoderma edule*; C. cra* = Crangon crangon*; D. lab* = Dicentrarchus labrax*; D. pug* = Diogenes pugilator*; E. vip* = Echiichthys vipera*; Gam. sp.* = Gammarus sp.*; I. lin* = Idotea linearis*; L. vul* = Loligo vulgaris*; M. bal* = Macoma balthica*; M. sla* = Mesopodopsis slabberi*; N. cir* = Nephtys cirrosa*; P. pla* = Pleuronectes platessa*; P. mic* = Pomatoschistus microps*; Pom. sp. = Pomatoschistus sp.; S. ker* = Schistomysis kervillei*; S. rho* = Scopthalmus rhombus*; S. ros* = Syngnathus rostellatus

Integrated approach towards the effect of *L. conchilega* on the food web structure

In general, at both locations the overlap of the standard ellipses for reef and control areas was high (Fig. 5) and found to be higher in spring (BMSM: 11.69‰^{2}; Boulogne: 9.05‰^{2}) than in autumn (BMSM: 7.83‰^{2}; Boulogne: 6.06‰^{2}) (Table 4). In spring, the sizes of the standard ellipse areas (SEA_c) of the reef communities was larger than those of the control communities (BMSM: reef = 15.99‰^{2}, control =14.58‰^{2}; Boulogne: reef = 14.50‰^{2}, control = 12.08‰^{2}) (Fig. 6, Table 4).

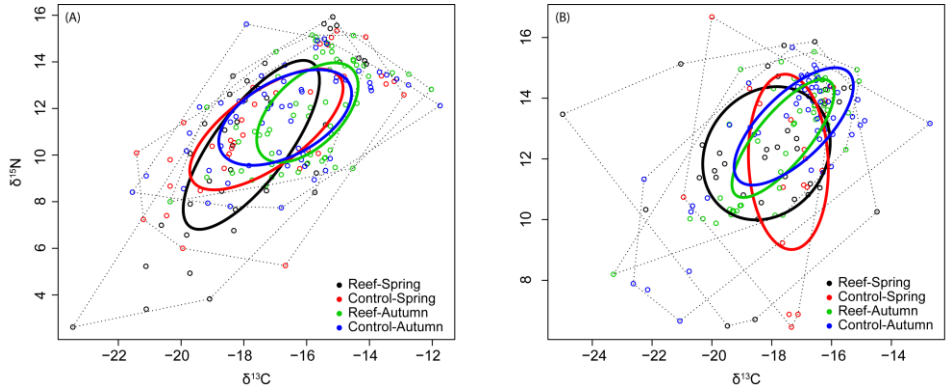

Figure 5. Biplot of $\delta^{13}C$ and $\delta^{15}N$ isotope values (‰) of all consumer individuals of the soft bottom intertidal areas of the Bay of the Mont Saint-Michel (A) and Boulogne-Sur-Mer (B). Solid lines enclose the standard ellipse area (SEA_C), representing the isotopic niche of consumer communities for different combinations of sampling area (reef vs. control) and period (spring vs. autumn). Dotted lines are the convex hulls representing the total niche width of the different consumer communities

The probability that SEA_B of the reef area is larger than the SEA_B of the control area in spring was 67.33% for the BMSM and 78% for Boulogne (Table 4). On the contrary, in autumn, the sizes of the standard ellipse areas (SEA_c) of the reef communities were smaller than those of the control communities (BMSM: reef = 9.13‰^{2}, control = 11.82‰^{2}; Boulogne: reef = 6.58‰^{2}, control = 8.71‰^{2}) (Fig. 6, Table 4). The probability that SEA_B of the reef area is larger than the SEA_B of the control area in autumn was 8.74% for the BMSM and 8.38% for Boulogne (Table 4). When comparing the two locations, the SEA_c for Boulogne were slightly smaller than those of the BMSM (Fig. 6).

Table 4. Bayesian Layman metrics (NR, CR, CD, MNND and SDNND), small sample size-corrected standard ellipse areas (SEA_C), and overlap in SEA_C ($‰^2$) between pairs of sampling area (reef vs. control) and period (spring vs. autumn) for the Bay of the Mont Saint-Michel (BMSM) and Boulogne-sur-Mer (Boulogne). The upper parts of the matrices show the overlap in SEA_C between pairs, while the lower parts show the Bayesian probability that the SEA of group 1 is smaller than the SEA of group 2. NR = $\delta^{15}N$ range, CR = $\delta^{13}C$ range, CD = mean distance to centroid, MNND = mean nearest neighbor distance, SDNND = standard deviation of the nearest neighbor distance. n = the number of individuals used to calculate the metrics.

								SEAc	Reef		Control	
		n	NR	CR	CD	MNND	SDNND	(‰²)	Spring	Autumn	Spring	Autumn
BMSM												
Reef	Spring	40	13.62	9.25	3.78	1.62	1.29	15.99	-	4.70	11.69	8.64
	Autumn	57	9.66	9.68	2.85	1.14	1.22	9.13	0.996	-	6.98	7.83
Control	Spring	35	11.81	10.38	3.70	1.78	1.46	14.58	0.673	0.017	-	10.88
	Autumn	36	10.69	11.52	3.14	1.17	1.18	11.82	0.924	0.087	0.812	-
Boulogne												
Reef	Spring	35	12.00	12.31	3.51	1.81	1.80	14.50	-	5.76	9.05	6.37
	Autumn	47	10.19	10.54	2.85	1.15	1.56	6.58	1.000	-	5.04	6.06
Control	Spring	20	11.10	7.68	3.20	2.07	1.71	12.08	0.780	0.020	-	5.79
	Autumn	56	12.11	11.21	3.25	1.27	1.53	8.71	0.987	0.084	0.846	-

Visual analysis of the credible intervals (Bayesian analogue of a confidence interval) of the Bayesian implementation of the Layman metrics showed for all 8 communities a high overlap in the *$\delta^{15}N$ range* (NR), the *$\delta^{13}C$ range* (CR) and the *standard deviation of the nearest neighbour distance* (SDNND) (Fig.7, Table 4). Credible intervals of the *mean distance to centroid* (CD) and the *mean nearest neighbour distance* (MNND) overlapped largely between reef and control communities, while slightly lower overlaps between spring and autumn communities were noted (Fig.7, Table 4).

Discussion

Notwithstanding the engineering effects of *L. conchilega* on the physical characteristics of the environment (Rabaut *et al.* 2009; Reise *et al.* 2009) and the subsequent alteration in the abundance and biomass of the primary producers and a broad spectrum of macrofaunal organisms (*e.g.* Zühlke *et al.* 1998; De Smet *et al.* 2013), the current study shows that the ecosystem engineering effect of *L. conchilega* does not directly affect the overall structure and isotopic niche of the food web of a soft-bottom intertidal ecosystem. However, an indirect engineering effect of the tubeworm on a minor fraction of the consumer taxa was observed.

Primary food sources

Primary food sources largely determine the food web structure by fuelling higher trophic levels in the system. Bulk organic matter (SPOM) and benthic diatoms (MPB_{diatom}) are the main food sources of the soft-bottom intertidal community, both in the presence and absence of *L. conchilega*.

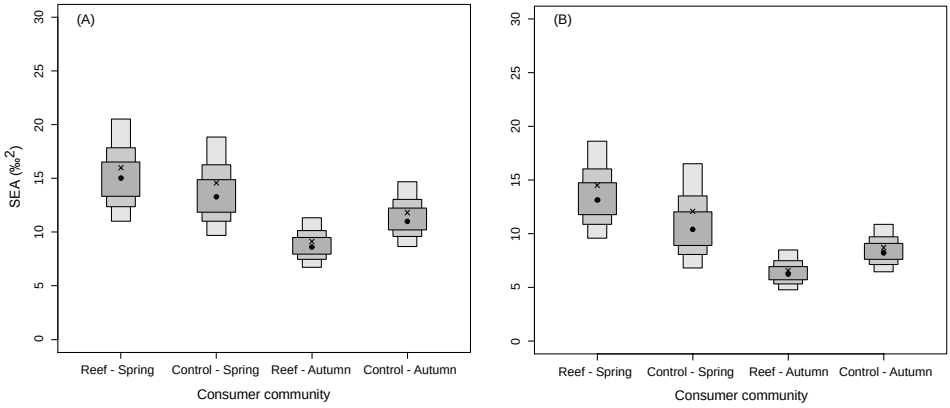

Figure 6. Density plots showing the credible intervals of the standard ellipse area (SEA) of consumer communities for different combinations of sampling area (reef vs. control) and period (spring vs. autumn) in the Bay of the Mont Saint-Michel (A) and Boulogne-Sur-Mer (B). Black dots are the mode of the SEA (‰²) while the shaded boxes represent the 50% (dark grey), 75% (lighter grey) and 95% (lightest grey) credible intervals. For comparison, small sample size-corrected SEA (SEA_C) are plotted as crosses

The tubes of *L. conchilega*, and animal tubes in soft bottom marine environments in general, can perturb the local flow conditions (*e.g.* Carey 1983), promoting a higher deposition rate of detrital organic matter (De Smet *et al.* unpublished) and an increased microbial colonisation, with bacteria considered as an important food source of deposit-feeding fauna (Eckman 1985). The $\delta^{13}C$ values of SPOM measured in this study (-22.23 ± 1.28‰) are in the same range as $\delta^{13}C$ values of pure phytoplankton in temperate coastal areas and estuaries (*e.g.* Gearing *et al.* 1984; Grall *et al.* 2006; Evrard *et al.* 2010). MPB_{diatom} on its turn is more enriched in ^{13}C (-13.14 ± 4.39‰) compared to SPOM, which is in line with the general trend that benthic algae in coastal environments have higher $\delta^{13}C$ values compared to phytoplankton (France 1995). Therefore, we suggest that the sampled SPOM is a mixture containing a rather small amount of locally produced MPB_{diatom} and predominantly water column-derived suspended POM. Stable isotope

values of the primary food sources were shown to be largely similar between the *L. conchilega* reef and control areas, implying an unaltered diversity of the most dominant primary resources in the presence of the tubeworm. Hence, the ecosystem engineering activity of *L. conchilega* does not directly modify the base of the food web. Isotope values differ rather seasonally: the $\delta^{13}C$ value of MPB_{diatom} in the BMSM is higher in spring than in autumn; however the opposite is the case for SPOM. Since changes in the amount of benthic diatoms among seasons are small (De Smet *et al.* 2015) and because they most probably only form a minor fraction of the bulk organic matter, its isotope signature seems to be masked by the high quantities of depleted POM in spring (De Smet *et al.* 2015). Conversely, in autumn the amounts of depleted POM are much lower (De Smet *et al.* 2015) leading to enriched benthic diatom-dominated bulk organic matter. Because SPOM is the most important carbon source for primary consumers in this study (Fig. 2 and 3), the soft-bottom intertidal food web seems to be mainly driven by carbon input from the water column, rather than by *in situ* primary production by benthic diatoms. This finding confirms the important trophic contribution of near shore phytoplankton to sandy beach macrofauna (Bergamino *et al.* 2011; Maria *et al.* 2012).

Consumers

Independent of the location, isotope values of primary food sources do not differ greatly among sampling areas and most consumer taxa co-occurring at both sampling areas exhibit similar isotope values, indicating that consumer taxa generally do not shift their diet when feeding in a *L. conchilega* reef. Moreover, the largely unchanged consumer diets are reflected in the almost invariable ranges in $\delta^{15}N$ and $\delta^{13}C$ and the highly similar isotopic niche widths of consumer communities of reef and control areas. This finding may not be surprising, keeping in mind that *L. conchilega* affects species which belong to the overall species pool of the habitat, rather than forming its own community (*i.e.* the so-called "Babushka" type of community structure Rabaut *et al.* 2007a; Van Hoey *et al.* 2008). Consequently, although the species densities differed, there's a high overlap in the species composition inside and outside a *L. conchilega* reef, possibly explaining the rather limited effect of a *L. conchilega* reef on the overall food web structure. Nonetheless, the high probability (at least 67%) that the isotopic niche width of the reef

communities is larger than the bare sand community in spring, in combination with the deviating isotope values of some consumer taxa in the reef areas implies that some consumers do shift their diet preference depending on the sampling area and the period. A diet shift is for instance the case for the brown shrimp *Crangon crangon*, which is one of the most abundant species in a *L. conchilega* reef (De Smet *et al.* 2015). When feeding in the *L. conchilega* reef in the BMSM during spring, *C. crangon* has a depleted $\delta^{13}C$ value and an enriched $\delta^{15}N$ value compared to a bare sand plot.

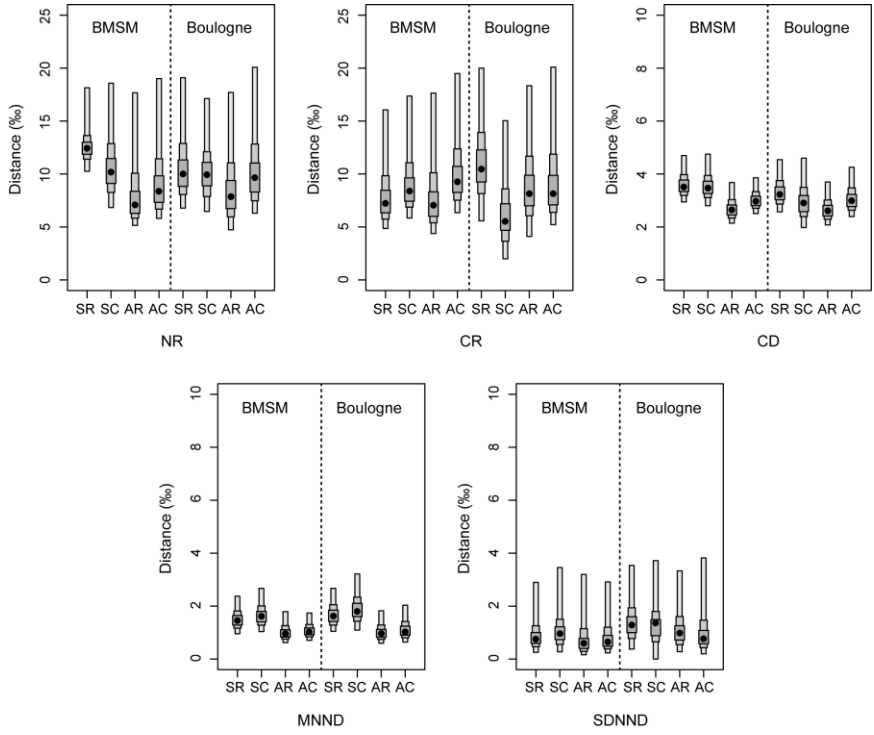

Figure 7. Density plot showing the uncertainty of the Bayesian Layman metrics (NR = $\delta^{15}N$ range, CR = $\delta^{13}C$ range, CD = mean distance to centroid, MNND = mean nearest neighbour distance, SDNND = standard deviation of the nearest neighbour distance) for different combinations of location (BMSM vs. Boulogne), sampling area (reef vs. control) and period (spring vs. autumn). Black dots represent the modes, while the shaded boxes represent the 50% (dark grey), 75% (light grey) and 95% (white) credible intervals. Note the different scales of distance (‰) for NR and CR vs. CD, MNND and SDNND. SR = spring-reef; SC = spring-control; AR = autumn-reef; AC = autumn-control

Deviating isotope values of taxa in the reef can be the result of the uptake of a specific food source or of the different community composition in the reef area. Firstly, the

specific food uptake can be the case if a consumer feeds on a more $\delta^{13}C$ enriched or depleted prey source which is merely available or more readily accessible in the reef due to the reef's specific habitat characteristics (*e.g.* shelter provision; Rabaut *et al.* 2007a). A prerequisite for this kind of change in the isotope values is that the consumer can circumvent the tidal cycle and remain in the reef for a longer period of time or that the consumer is able to find its way back to the reef. The former is likely for macrobenthic animals and consumer taxa showing burying behaviour when the water retreats such as *C. crangon*, (pers. obs. in experimental setups, Janssen and Kuipers 1980). More mobile, non-burrowing epifauna with deviating isotope values in the reef, such as *Loligo vulgaris* and *Syngnathus rostellatus*, are believed to be able to reoccupy their position in the reef at high tide. Secondly, the different composition of the associated reef fauna might affect the food selectivity of consumers. The higher number of trophic groups in the reef area and the increased spread of functional groups over trophic groups suggest a slightly different food selectivity in the reef and hence a decrease in the competition between consumers which is supposed to be beneficial in the densely populated reef areas. Despite the reported locally increased species richness in a reef (cfr. the "Babushka" effect; Rabaut *et al.* 2007a; Van Hoey *et al.* 2008), the number of taxa between reef and control areas was hardly different in this study. Nonetheless, standardised sampling techniques were used and we believe that the gathered data veraciously reflect the observed food web structure.

While most taxa do not show a diet shift in the reef, a more in-depth view reveals that there might be an indirect engineering effect of *L. conchilega* on a minor fraction of the consumer taxa owing to a specific food uptake and/or the different community composition in the reef area.

Isotopic niche width was not different among sampling areas (reef *vs.* control), but differences among periods (spring *vs.* autumn) were shown to be slightly larger, indicating that the isotopic niche width of the consumer communities among periods is less alike than the isotopic niche width of the communities among sampling areas. A shift in the diet of the consumer taxa from spring to autumn and vice versa most reasonably explains the observed differences (Dubois and Colombo 2014). Species

packing and hence trophic redundancy among sampling areas (as measured by MNND and SDNND metrics) seems not to be affected, pointing to an unaltered stability of the food web in the presence of *L. conchilega*. However, based on the increased sediment stability in the presence of *L. conchilega* (Rabaut *et al.* 2009; Passarelli *et al.* 2012), a stabilizing effect of the ecosystem engineer on the food web base was expected. The observed unaltered stability can be related to the dependence of the food web on water column-derived primary production (SPOM) rather than *in situ* primary production from the sediment. Comparison of trophic redundancy and overall species packing among periods reveals that the autumn communities showed an increase (by the lower values of MNND and to some extent by the lower values of CD; Brind'Amour and Dubois 2013), compared to their counterparts in spring. Hence, food web stability is slightly higher in autumn compared to spring.

Linking ecosystem engineering and food webs

Although integrated studies on ecosystem engineering and food web structure are rare in marine research, the outcomes of our study are largely in line with existing knowledge. Rigolet *et al.* (2014b) investigated changes in the benthic food web structure of an *Amphiura filiformis* habitat colonised by the engineering tubicolous amphipod *Haploops nirae*. Despite altering local sediment features and positively affecting the local biodiversity and associated species assemblages (Rigolet *et al.* 2014a), *H. nirae* did not affect the food web structure as based on ranges in $\delta^{13}C$ and $\delta^{15}N$. Similarly, Baeta *et al.* (2009) found neither differences in the planktonic nor the benthic food web structure between a site dominated by eelgrass *Zostera noltei* and bare sediment. A study by Botto *et al.* (2005) shows that the burrowing crab *Neohelice granulata*, an engineer in SW Atlantic coastal areas, modifies $\delta^{15}N$ values of sediments and primary producers by 3 to 7‰. Some consumers associated with the sedimentary environment reflected the enriched N values, but the overall food web structure in areas with and without the crab however remained largely unaltered.

Apart from the well-documented effects of an ecosystem engineer on its environment and on the composition of a community, the results of the current and previous studies did not show a global impact of the presence of ecosystem engineers on the marine

food web structure; contrasting recently formulated hypotheses (Sanders *et al.* 2014). As opposed to the strong impact of *L. conchilega* on the benthic assemblage, the ecosystem engineer's influence on the water column is probably too limited to substantially stir the global structure of the soft-bottom intertidal food web, which is mainly driven by water-column derived primary production. Nevertheless, an indirect engineering effect of the tubeworm on a minor fraction of the consumer taxa was observed, which indicates that a more detailed species-specific approach might be better suited to reveal difference in the food web structure in the presence of an ecosystem-engineered *L. conchilega* reef.

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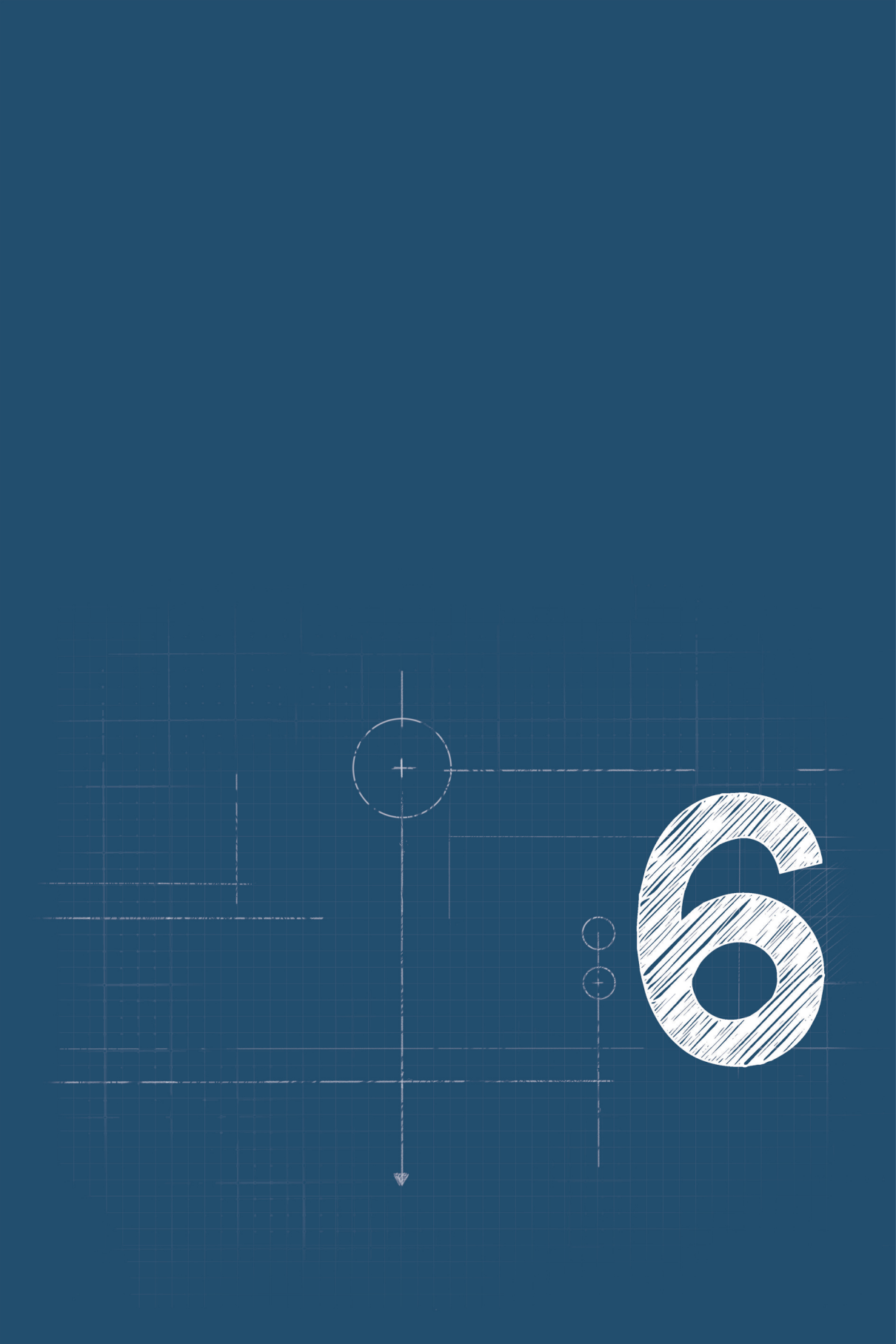
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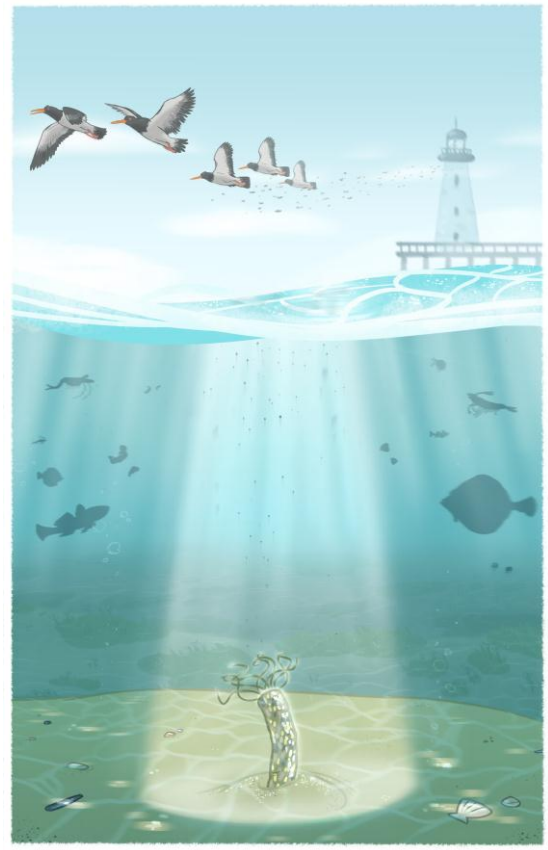
Chapter 6

Lanice conchilega structures carbon flows in soft-bottom intertidal areas



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Abstract

Biogenic reefs constructed by the tube-building ecosystem engineer *Lanice conchilega* (Terrebilidae, Polychaeta) have profound structuring impacts on the benthic environment by altering the biogeochemical and physical properties of the sediment. Their role in the food web dynamics of soft-bottom intertidal areas is less well studied and hitherto considered to be rather limited. This study provides new insights in the functioning of *L. conchilega* reefs in intertidal sediments by quantifying the carbon flows in the food webs in the presence and absence of the tubeworm using linear inverse models (LIMs). The food web models were based on an empirical dataset from two study sites and two time periods, consisting of biomass and stable isotope data, and general physiological constraints from the literature. The carbon input into reef food webs (191 ± 50 mmol C m^{-2} d^{-1}) is about 40 times higher compared to bare sand areas (5 ± 2 mmol C m^{-2} d^{-1}) and is mainly derived from organic matter (OM) in the water column. The tubeworm traps the OM which is produced in an area at least 15 times larger than the reef itself. Although most of the OM input towards these reefs is consumed by suspension feeding macrofauna, particularly *L. conchilega* itself, the worm is not an important source of carbon for other macrofaunal organisms. The structures created by *L. conchilega* rather act as a trap of OM, resulting in overall high macrofaunal biomass in the presence of the tubeworm, and much more diverse food webs.

Keywords: linear inverse model, biogenic reef, food web, network analysis, stable isotopes, *Lanice conchilega*, ecosystem engineering

Introduction

Ecosystem engineers are organisms that directly or indirectly modulate the availability of resources to other species by reshaping the landscape (Jones *et al.* 1994). Biogenic reefs constructed by these ecosystem engineers are some of the most striking structures in soft-bottom intertidal areas. A prime example are the aggregations of the tubeworm *Lanice conchilega* (Polychaeta, Terebellidae), reaching up to several thousands of individuals per square meter (Van Hoey *et al.* 2006) and as such called *Lanice conchilega* reefs (Rabaut *et al.* 2009). In Europe, where the largest populations of this tube-dwelling polychaete are found, *L. conchilega* colonises a wide range of intertidal and subtidal sediments down to a depth of approximately 1900 m (Hartmann-Schröder 1996). The largest reefs are found in the Wadden Sea (Petersen and Exo 1999) and in France, especially in the Bay of the Mont Saint-Michel (Godet *et al.* 2008).

Lanice conchilega reefs have profound structuring impacts on the benthic environment by altering the biogeochemical (Forster and Graf 1995; Braeckman *et al.* 2010) and physical properties of the sediment (*e.g.* Degraer *et al.* 2008; Rabaut *et al.* 2009; Borsje *et al.* 2014). As a result, *L. conchilega* affects the community composition, abundance and species richness within the reef (Zühlke *et al.* 1998; Rabaut *et al.* 2007a; Godet *et al.* 2008; Braeckman *et al.* 2011b; De Smet *et al.* 2013; Rabaut *et al.* 2013). Moreover, intertidal *L. conchilega* reefs are important from a conservation point of view due to their central role in the link between benthic, pelagic and air-borne parts of the intertidal food web (De Smet *et al.* 2013; De Smet *et al.* 2015). Notwithstanding their structuring role on the meio- and macrofauna, these reefs seem to have a minor effect on the overall structure of the soft-bottom intertidal food web. Based on stable isotope analysis, the consumer community in the presence and absence of *L. conchilega* showed very similar isotopic niche widths, implying that consumers hardly change their diet when feeding in a *L. conchilega* reef (De Smet *et al.* in press). Also the stability of the food web in the presence of *L. conchilega,* as evaluated based on the density of species packing and hence the trophic redundancy, remained unaltered (De Smet *et al.* in press).

Whereas the above study looked at qualitative aspects, another important aspect of food web research comprises the quantification of food web flows, which yields a more

realistic approximation of complex food webs (De Ruiter *et al.* 1995; Woodward *et al.* 2005). The *in situ* quantification of food web flows is a labour-intensive task (Berlow *et al.* 2004), but the development of linear inverse modelling has been a big step forward in food web research. Linear inverse modelling is a data assimilation method allowing the integration of carbon processes (*e.g.* respiration) and biomass and stable isotope data to quantify elemental flows in food webs (Vézina and Platt 1988; van Oevelen *et al.* 2006b; van Oevelen *et al.* 2010). By merging a variety of traditional data types and currently underexploited data sources, linear inverse models (LIMs) are a powerful tool to quantitatively reconstruct pathways in systems as small as a single cell up to entire food webs. This feature makes LIMs an effective tool to bridge the gap between incomplete and uncertain empirical data on natural food webs and the analysis of food web structures (van Oevelen *et al.* 2010).

Despite the seemingly limited effect of *L. conchilega* on the food web topology (De Smet *et al.* in press), a quantification of the flows in a food web dominated by *L. conchilega* has never been done, nor has the magnitude of the carbon flows between the different food web compartments been quantified. Nevertheless, given the impact of *L. conchilega* on the biogeochemical and physical aspects of its environment and on the macrofaunal community, it can be expected to alter the food web flows in the intertidal. This study develops LIMs to present a comprehensive overview of the carbon cycling and dynamics of a soft-bottom intertidal food web in the presence and absence of ecosystem-engineered *L. conchilega* reefs, focussing on the macrofaunal part of the food web. A selection of network indices, representing the complex interactions between the compartments, is used to compare the different food webs. Moreover, in order to address temporal and spatial variation in the food web structure, two study sites and two time periods were taken into account.

Material & Methods

Study site and food web structure

The two study sites are located along the French coast of the English Channel and were selected because of their extensive and well-established intertidal *Lanice conchilega*

reefs. The Bay of the Mont Saint-Michel (BMSM) is a large-scale intertidal sand flat in the Normand-Breton Gulf (48°39.70'N - 01°37.41'W; Lower Normandy, France), while Boulogne-sur-Mer (further referred to as Boulogne) is a small soft-bottom intertidal area in the Nord-Pas de Calais region (50°44.01'N - 01°35.15'E; Northern France) (Fig. 1). At both locations, an area dominated by a *L. conchilega* reef and a bare sediment area were chosen for reconstructing the carbon cycling through the macrofaunal part of the food web. A separate analysis was performed for two time periods, spring and autumn. Consequently, eight different food webs were quantified.

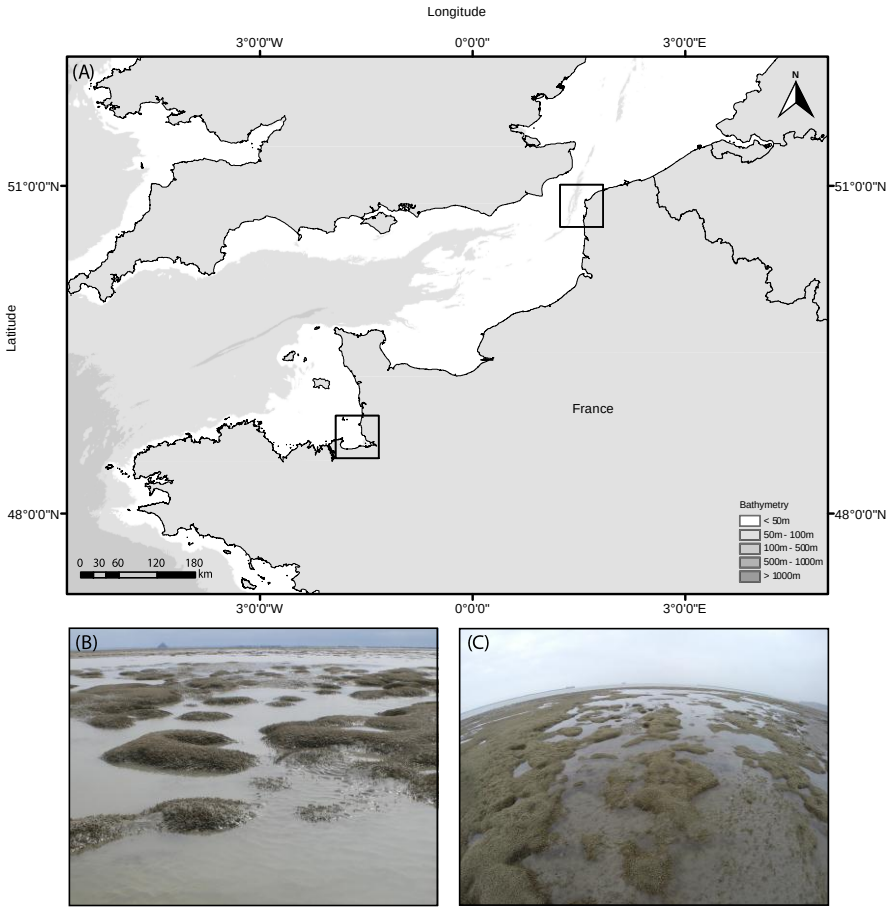

Figure 1. The two study sites along the French side of the English Channel: Boulogne-sur-Mer (Boulogne; upper square) and the Bay of the Mont Saint-Michel (BMSM; lower square) (A), and the Lanice conchilega *reef in the intertidal area of the BMSM (B) and Boulogne (C)*

This study focusses on the bentho-pelagic community of the soft-bottom intertidal food web (De Smet *et al.* 2015). The most abundant species, ranging from smaller polychaetes up to fish, were included as separate compartments interconnected by flows (Fig. 2 shows a simplified version of the complex overall food web used in this study).

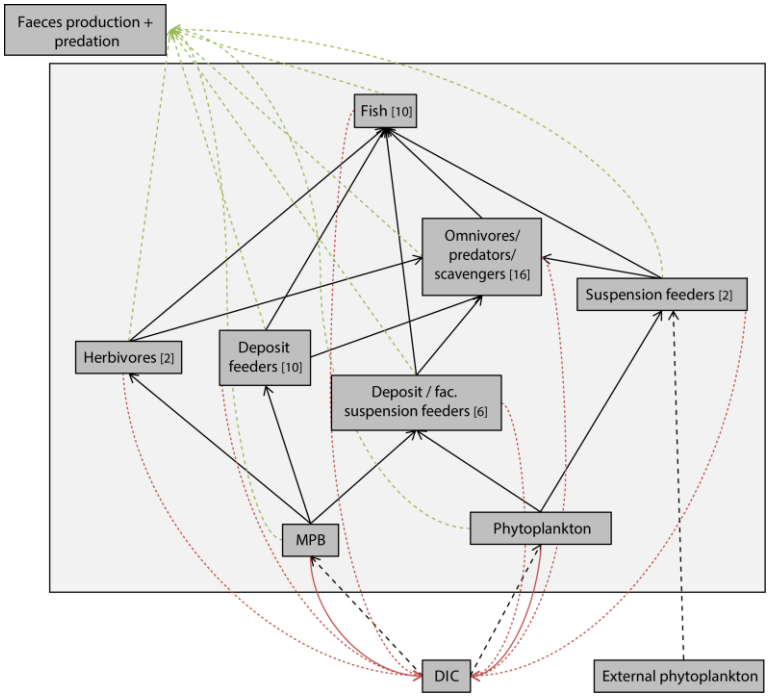

Figure 2. Simplified schematic representation of the overall complex topological food web on which the LIMs are based. Compartments inside the box are included in the model, while compartments outside the box were not explicitly modelled. Links between the compartments were based on literature and stomach content analysis of fish. For simplicity, species where lumped in functional groups in the scheme, however single species compartments were used in the model (the number of species included in every functional group is displayed between brackets). Black dashed arrows represent the external input to the model, while red dashed arrows represent the loss of carbon from the food web as a result of respiration and green dashed arrows the production of faeces + predation by animals not included in the model. MPB = microphytobenthos, DIC = dissolved inorganic carbon

Sedimentary microphytobenthos and water-column derived phytoplankton constitute the inputs to the food webs; they are fed upon by detritus- and suspension-feeding macrofauna. Connections between the macrofaunal compartments were defined based on diet information from literature; FishBase (Froese and Pauly 2015) as well as results from stomach content analysis (Verhelst 2013) were used to define flows to and among

fish species, while Fauchald and Jumars (1979), De Smet *et al.* (in press) and the World Register of Marine Species (WoRMS) were used to define flows to and among all other macrofaunal compartments. Loss of carbon from the food webs includes macrofaunal mortality and faeces production, macrofaunal respiration, and predation due to animals not accounted for in the model (*e.g.* predation by wading birds and seals). The *a priori* defined compartments interconnected by flows constitute the overall topological food web model on which the linear inverse model for the eight food webs is based (Fig. 2).

Data availability

Site-specific empirical data and literature data on carbon stocks and process rates were implemented in the food web model. The empirical data included biomasses and $\delta^{13}C$ stable isotope signatures of primary food sources and consumer species. In order to further constrain the carbon flows in the food web, primary production rates, respiration rates, assimilation efficiencies and net growth efficiencies of macrofaunal compartments were taken from the literature.

Empirical data

At both locations, quantitative sampling of the primary food sources (microphytobenthos and phytoplankton) and the macro-, hyper- and epibenthic fauna inhabiting a *L. conchilega* reef area and a bare sand area took place in spring (from 7th till 13th of March in the BMSM and from 22nd till 25th of March in Boulogne) and autumn 2012 (from 17th till 21st of September in BMSM and from 15th till 18th of October in Boulogne). The carbon pool (in mmol C m^{-2}) in microphytobenthos (MICPHY) and phytoplankton (PHYTOP) was calculated by converting the amount of Chl *a* to carbon equivalents, assuming a C:Chl *a* ratio of 40 (Stephens *et al.* 1997), and subsequently to molar units. Macro-, hyper- and epibenthic organisms were collected, identified to species level and counted. Species that could not be distinguished with certainty were grouped at the genus level (*see* De Smet *et al.* (2015) for a detailed description of the sampling design and processing). Wet mass of the organisms was determined and subsequently converted to dry mass and carbon units (mmol C m^{-2}) by using taxon-specific conversion factors from Brey (2001) (Appendix 1). Stable isotope data ($\delta^{13}C$)

were available for the primary food sources and the majority of the faunal species (De Smet *et al.* in press) (Appendix 2). If the isotope value of a species was not available for a sampling area (reef and bare sand), a season (spring and autumn) or a location (BMSM and Boulogne), the isotope value of that species from another sampling area, season or location was assumed (Appendix 2). If isotope data of a species was completely lacking, the isotope values of a taxonomically related species were adopted (*e.g.* for bivalves: the $\delta^{13}C$ value of *Macoma balthica* was adopted for *Tellina tenuis*), or the isotope value of the reference situation of a ^{13}C pulse-chase experiment, conducted for the site of Boulogne, was used (De Smet *et al.* under review).

Literature data

Minimum and maximum bounds on the production rates of microphytobenthos per season for the BMSM were taken from gross primary production (GPP) measurements in the intertidal area of the BMSM (Davoult *et al.* 2009) (Table 1). As no literature data on the GPP of microphytobenthos for the intertidal area of Boulogne were available, the GPP in the BMSM was used for Boulogne (Table 1). Maximum bounds on the seasonal production rate of phytoplankton were obtained from GPP measurements in the bay of Marennes-Oléron along the Atlantic coast of France (Struski and Bacher 2006), and assumed to be the same for the areas of the BMSM and Boulogne (Table 1). Since the primary production measured by Struski and Bacher (2006) was largely the same in spring and autumn, a constant GPP was used for both seasons. The minimum and maximum respiration rates of the primary producers were set at 5% and 30% of the GPP respectively (Vézina and Platt 1988) (Table 1).

Macrofaunal respiration was implemented as the sum of maintenance respiration and growth respiration (Soetaert and van Oevelen 2009). Maintenance respiration was taken as 1% of the biomass per day for smaller macrofaunal organisms (Polychaeta, Isopoda, Mysida and Amphipoda) (Fenchel 1982; Nielsen *et al.* 1995) and 0.1% for larger invertebrates (Decapoda, Bivalvia and Cephalopoda) and fish (*e.g.* Hepher *et al.* 1983). Subsequently, the maintenance respiration was corrected for temperature with a temperature limitation factor (Tlim) based on a Q_{10} of 2 (van Oevelen *et al.* 2012). The

total respiration was estimated from biomass by using an allometric formula for shallow water organisms at a 15-20°C temperature range (Mahaut *et al.* 1995):

$$r = 0.0174.W^{-0.156}$$

with *r* being the biomass-specific respiration rate (d^{-1}) and *W* the individual biomass (mg C ind^{-1}). The respiration rate (*R*) of each species was calculated by multiplying its biomass with the biomass-specific respiration rate and converting it to carbon units (mmol C m^{-2}). The lower and upper boundaries on the respiration rate were set as *R*/2 and 2×*R* respectively (van Oevelen *et al.* 2009).

Table 1. Constraints imposed on processes implemented in the macrofaunal food webs. The minimum (lower boundary) and maximum (upper boundary) values are implemented as inequalities in the linear inverse model. Sources represent the literature from which the data was obtained.

Process	Unit	Lower boundary	Upper boundary	Sources
Gross primary production (GPP)	mmol C m^{-2} d^{-1}			
phytoplankton (autum)		–	9.99	Struski and Bacher (2006)
phytoplankton (spring)		–	9.99	Struski and Bacher (2006)
microphytobenthos (autumn)		19.98	39.96	Davoult *et al.* (2009)
microphytobenthos (spring)		24.98	44.96	Davoult *et al.* (2009)
Respiration	mmol C m^{-2} d^{-1}			
phytoplankton		0.05 * GPP	0.30 * GPP	Vézina and Platt (1988)
microphytobenthos		0.05 * GPP	0.30 * GPP	Vézina and Platt (1988)
Assimilation efficiency (*AE*)	–			
L. conchilega		0.71	0.77	Buhr 1976
Fish		0.40	0.80	Jobling (1994), Hendriks (1999)
Other macrofauna		0.58	0.85	Schroeder (1981), Jordana *et al.* (2001)
Net growth efficiency (*NGE*)	–	0.30	0.70	Calow (1977), Banse (1979), Hendriks (1999)

The assimilation efficiency (*AE*) is the ratio of assimilation (= production + respiration) to consumption and is independent of body size (Banse 1979; Hendriks 1999). Lower and upper boundaries on the *AE* were between 71% and 77% for *L. conchilega* (Buhr 1976), between 40% and 80% for fish species (Jobling 1994; Hendriks 1999), and between 58% and 85% for all other macrofaunal organisms (Schroeder 1981; Jordana *et al.* 2001) (Table 1). Finally, the net growth efficiency (*NGE*) is the ratio of production (*i.e.* growth) to assimilation. The *NGE* is independent of body size and lower and upper boundaries on the faunal *NGE* were set between 30% and 70% (Calow 1977; Banse 1979; Hendriks 1999) (Table 1).

Linear inverse model formulation

Based on the overall topological food web, an inverse model was developed. From the mathematical point of view, an inverse model contains data on carbon flows in the food web casted in matrix notation as a set of linear equality (1) and inequality equations (2):

$$\mathbf{A} \cdot \mathbf{x} = \mathbf{b} \quad (1)$$

$$\mathbf{G} \cdot \mathbf{x} \geq \mathbf{h} \quad (2)$$

in which **x** is a vector with *N* unknown food web flows. Each row in matrix **A** is a mass balance or data point expressed as a linear combination of the food web flows, where the corresponding rate of change of a compartment (for mass balances) or numerical value (for data equalities) is given in vector **b**. **h** is a vector containing values of biological constraints and the constraint coefficients, signifying whether and how much a flow contributes to the constraint, are given in matrix **G** (Vézina and Platt 1988). Quantitative, site-specific data (*i.e.* the biomass and $\delta^{13}C$ values of the different compartments) were added to the model as equality equations since they are all linear functions of the flows. Absolute values of the lower and upper bounds of the literature data (production rates, assimilation efficiencies, net growth efficiencies and respiration rates) were added to the model as inequalities since they are used to constrain single flows or linear combinations of flows to biologically realistic values. The complete model consists of 727 flows, 48 mass balances (*i.e.* one for each compartment), 46 data equalities and 142 data inequalities.

As in the majority of LIMs, the food webs in this study are under-sampled and the number of equalities (48 + 46) is insufficient to balance the number of unknown flows. Therefore, the LIM is under-determined and the matrix equations have an infinite number of solutions (van Oevelen *et al.* 2010). Solving this problem can be done in three ways: (1) upgrading lower-quality literature data to high-quality data that can be incorporated as fixed equalities; (2) singling out one "best" solution (*i.e.* food web structure) based on the assumption of parsimony or simplicity; or (3) quantifying uncertainty, *i.e.* using a likelihood approach which generates a large set of possible solutions, or determining flow ranges. Because of the exceptionally high amount of food

web compartments in this study, the parsimony approach was used, together with range estimation. The most parsimonious solution is defined as the flow vector x that has the smallest sum of squared flow values (Vézina and Platt 1988). Additionally, by means of a range estimation procedure, the minimum and maximum values of each flow in the solution space was determined (Klepper and Van de Kamer 1987). The freely available software package *LIM* (Soetaert and van Oevelen 2008) was used to solve the LIMs in R (Version 3.1.2) (R Development Core Team 2015).

Network indices

In order to compare the complex interactions between compartments for the different food webs, several network indices were calculated from the output of the parsimony approach: total system throughput ($T_{..}$, the sum of all flow magnitudes in a network), total number of links (L_{tot}), the average link weight ($\overline{T}_{ij}$), the connectance (C) and the average mutual information index (*AMI*, the average amount of constraint placed upon an arbitrary flow anywhere in the network). The R-package *NetIndices* (Kones *et al.* 2009) was used to calculate the network indices in R. A summary of the nomenclature (Table 2) and the equations (Table 3) is given in this manuscript, but for a detailed overview see Ulanowicz (2004) and Kones *et al.* (2009).

Table 2. Nomenclature of the symbols used in the calculation of the network indices (Table 3).

Term	Description
n	Number of internal compartments in the network, excluding 0 (zero), $n + 1$ and $n + 2$
$j = 0$	External source (*i.e.* dissolved inorganic carbon and detritus input)
$j = n + 1$	Useable export from the food web (*i.e.* secondary production)
$j = n + 2$	Unusable export from the food web (*i.e.* respiration and detritus production)
T_{ij}	Flow from compartment j to i where j represents the columns of the flow matrix and i the rows
$T_{i.}$	Total inflows to compartment i
$T_{.j}$	Total outflows from compartment j
T_i	Total inflows to compartment i excluding inflow from external sources
T_j	Total outflows from compartment j excluding outflow to external sources

Table 3. Algorithms for the calculation of the network indices (see Table 2 for a nomenclature of the symbols).

Index name	Code	Formula
Total system throughput	$T..$	$\sum_{i=1}^{n+2}\sum_{j=0}^{n} T_{ij}$
Number of links	L	$\sum_{i=1}^{n+2}\sum_{j=0}^{n}(T_{ij}>0)$
Average link weight	$\overline{T}_{ij}$	$T../L$
Connectance	C	$\frac{L_{int}}{n(n-1)}$ where $L_{int}=\sum_{i=1}^{n}\sum_{j=1}^{n}(T_{ij}>0)$
Average mutual information index	AMI	$\sum_{i=1}^{n+2}\sum_{j=0}^{n}\frac{T_{ij}}{T..}\log_2\frac{T_{ij}\,T..}{T_{i.}T_{.j}}$

Results

Biomass data

The mean total biomass (± SE) in a *L. conchilega* reef area (18969 ± 3992 mmol C m^{-2}) was two orders of magnitude higher than the mean total biomass in a bare sand area (180 ± 56 mmol C m^{-2}) (Appendix 1). The macrofaunal biomass in the reef areas was largely dominated by suspension feeders (BMSM: 85 ± 4%, Boulogne: 82 ± 13%). Main suspension feeders in the BMSM included *L. conchilega* (37 ± 1%) and the bivalve *Cerastoderma edule* (48 ± 4%). The remaining compartments constituted 15 ± 4% of the total macrofaunal biomass. In the Boulogne reefs, *L. conchilega* made up the majority of the macrofaunal biomass (82 ± 13%), which was further complemented with the predator *Carcinus maenas* (17 ± 13%). The remaining compartments constituted 2 ± 1% of the total macrofaunal biomass. In the bare sand areas of the BMSM, carbon stocks were much more variable and highest for the bivalves *C. edule* (33 ± 33%), *M. balthica* (29 ± 10%) and *Tellina tenuis* (12 ± 7%), and the polychaetes *Arenicola marina* (8 ± 8%) and *Nephtys cirrosa* (6 ± 0.3%). The remaining compartments accounted for 12 ± 8% of the total biomass in the bare sand areas of the BMSM. Carbon stocks in de bare sand areas of

Boulogne were dominated by *C. maenas* (48 ± 42%) and *N. cirrosa* (33 ± 26%). The remaining compartments accounted for 19 ± 16% of the total biomass.

Food web structure and model output

Macrofaunal species richness (number of compartments) was higher in reef areas (32 ± 4 species) as compared to bare sand areas (24 ± 4 species). Differences in the food web structure become apparent when the carbon flows are plotted in a circular fashion (Figure 3 and 4; Appendices 3 and 4).

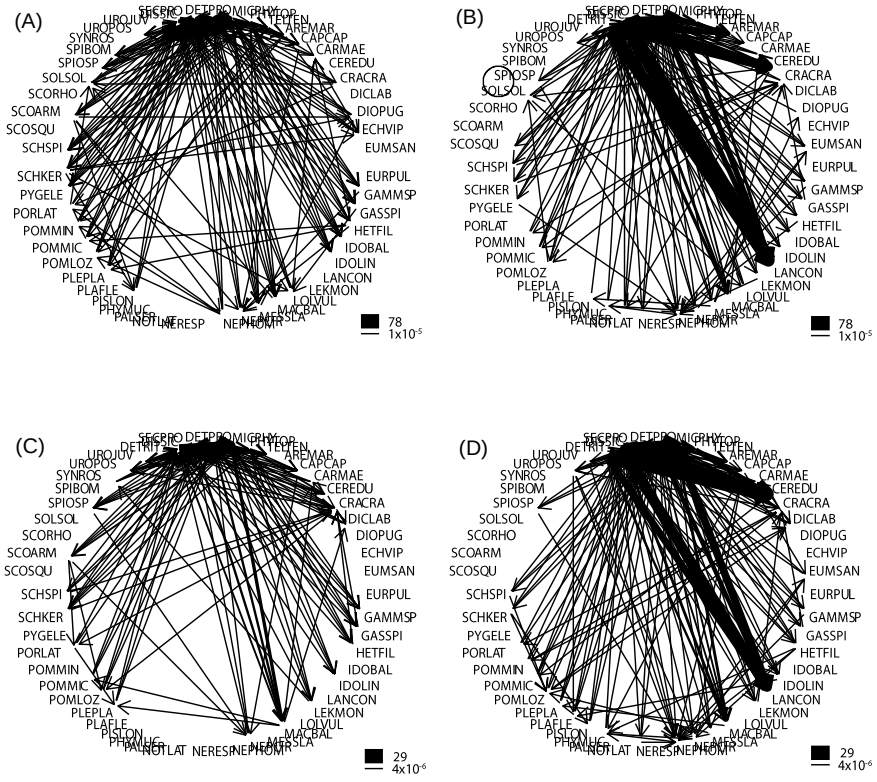

Figure 3. Food web flows (mmol C m^{-2} d^{-1}) returned by the linear inverse food web models for the soft-bottom intertidal area of the Bay of the Mont Saint-Michel (BMSM): Bare sand-Autumn (A), Reef-Autumn (B), Bare sand-Spring (C), Reef-Spring (D). Note the different magnitude of the plotted flows between seasons: autumn: $1x10^{-5}$ - 78 mmol C m^{-2} d^{-1} (A-B); spring: $4x10^{-6}$ - 29 mmol C m^{-2} d^{-1} (C-D). Circular flows indicate cannibalism. See Appendices 1 and 2 for abbreviations of the food web compartments. Other abbreviations: DETPRO = external outflow to detritus, DETRIT = external inflow of organic matter, DISSIC = dissolved inorganic carbon, SECPRO = external outflow of secondary production. Minimum and maximum values as well as the parsimonious solution of the most important food web flows are given in Appendix 3

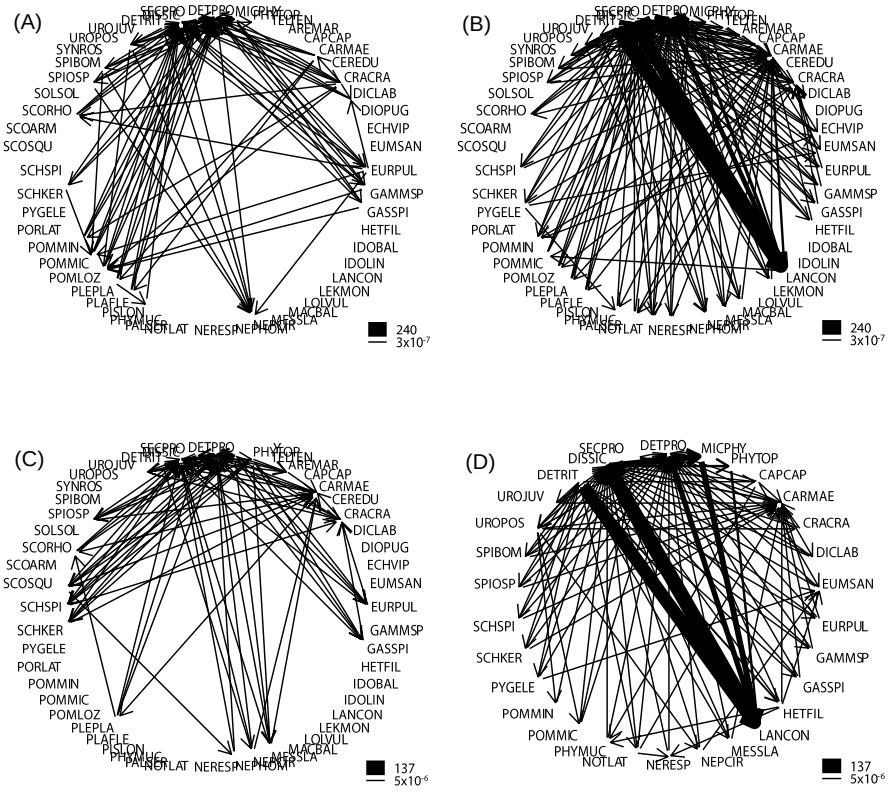

Figure 4. Food web flows (mmol C m^{-2} d^{-1}) returned by the linear inverse food web models for the soft-bottom intertidal area of Boulogne-sur-Mer (Boulogne): Bare sand-Autumn (A), Reef-Autumn (B), Bare sand-Spring (C), Reef-Spring (D). Note the different magnitude of the plotted flows between seasons: autumn: $3x10^{-7}$ - 240 mmol C m^{-2} d^{-1} (A-B); spring: $5x10^{-6}$ - 137 mmol C m^{-2} d^{-1} (C-D). See Appendices 1 and 2 for abbreviations of the food web compartments. Other abbreviations: DETPRO = external outflow to detritus, DETRIT = external inflow of organic matter, DISSIC = dissolved inorganic carbon, SECPRO = external outflow of secondary production. Minimum and maximum values as well as the parsimonious solution of the most important food web flows are given in Appendix 4

The main difference between reef and bare sand food webs was the important role of *L. conchilega* in the reef areas, mainly complemented by the compartments of the bivalve *C. edule* in the food webs of the BMSM, while *C. maenas* had a prominent role in the food webs of Boulogne. In the bare sand areas, the mean total carbon ingested by the macrofaunal community was 5 ± 2 mmol C m^{-2} d^{-1} and was partitioned among the sources as 19 ± 5% *in situ* produced phytoplankton, 58 ± 13% microphytobenthos and 24 ± 13% external phytoplankton (Table 4). In the reef areas, mean total carbon ingested by the macrofaunal community was much higher (191 ± 50 mmol C m^{-2} d^{-1}). Moreover, in

contrast to bare sand areas, external phytoplankton accounted for 73 ± 5% of the carbon uptake by macrofauna, while *in situ* produced phytoplankton (7 ± 2%) and microphytobenthos (20 ± 3%) were less important (Table 4).

Table 4. Total ingested carbon (in mmol C m^{-2} d^{-1}) and its partitioning among primary food sources for the different food web models.

	BMSM				Boulogne			
	Autumn		Spring		Autumn		Spring	
	Control	Reef	Control	Reef	Control	Reef	Control	Reef
In situ phytoplankton	1.39 (18 %)	9.49 (5 %)	2.21 (27 %)	9.49 (13 %)	0.04 (6 %)	9.49 (3 %)	0.45 (24 %)	9.49 (5 %)
Microphytobenthos	6.49 (82 %)	36.55 (20 %)	5.70 (70 %)	18.56 (26 %)	0.41 (58 %)	38.96 (12 %)	0.42 (22 %)	43.71 (23 %)
External phytoplankton	0.00 (0 %)	138.40 (75 %)	0.26 (3 %)	44.62 (61 %)	0.26 (36 %)	270.60 (85 %)	1.05 (55 %)	136.79 (72 %)
Total carbon ingested	7.88	184.44	8.18	72.67	0.71	319.05	1.92	189.99

The majority of the total organic carbon in the reef areas was consumed by *L. conchilega* and ranged between 37% in the BMSM in spring and 94% in Boulogne in spring. Only in the reef area of the BMSM during spring, a larger part of the organic carbon (51%) was ingested by *C. edule*. Notwithstanding the important carbon flow from primary food sources to *L. conchilega*, the carbon throughput from the tubeworm to other macrofaunal compartments equalled zero for all reef food webs. The total respiration of the bare sand community was 6 ± 2 mmol C m^{-2} d^{-1} in the BMSM and 6 ± 3 mmol C m^{-2} d^{-1} in Boulogne. Total respiration of the reef community was estimated to be 99 ± 46 mmol C m^{-2} d^{-1} in the BMSM, and 193 ± 48 mmol C m^{-2} d^{-1} in Boulogne. *L. conchilega* performed 46 ± 7% of the total respiration in the reef of the BMSM and 89 ± 5% in the reef of Boulogne.

Network indices

The network indices total system throughput (*T..*), total number of links (L_{tot}), connectance (*C*), average link weight ($\overline{T}_{ij}$), and average mutual information (*AMI*) were calculated for the 8 different food webs (Table 5). The mean *T..* (± SE) was considerably higher in a *L. conchilega* reef food web compared to a bare sand food web, both for the BMSM (reef: 305 ± 118 mmol C m^{-2} d^{-1}, bare sand: 59 ± 5 mmol C m^{-2} d^{-1}) and Boulogne (reef: 578 ± 136 mmol C m^{-2} d^{-1}, bare sand: 49 ± 7 mmol C m^{-2} d^{-1}), although the number of links, L_{tot}, was virtually the same in the bare sand (137 ± 20) and reef (135 ± 6) food

webs of the BMSM, but conspicuously higher in the reef (121 ± 23) compared to the bare sand (79 ± 4) food webs of Boulogne.

Table 5. Overview of the calculated network indices for the reef and bare sand food webs of the Bay of the Mont Saint-Michel (BMSM) and Boulogne for spring and autumn, based on the parsimony approach. T.. = total system throughput (mmol C m^{-2} d^{-1}), Ltot = total number of links, C = connectance, $\overline{T}_{ij}$ = average link weight, and AMI = average mutual information. The trophic level and omnivory indices are displayed as the mean (±SE) of the single species values within each food web.

	BMSM				Boulogne			
	Autumn		Spring		Autumn		Spring	
	Control	Reef	Control	Reef	Control	Reef	Control	Reef
$T..$	53	423	64	187	42	714	55	441
L_{tot}	156	129	117	141	82	144	75	98
C	0.063	0.026	0.074	0.051	0.074	0.034	0.125	0.045
$\overline{T}_{ij}$	0.34	3.28	0.55	1.33	0.51	4.96	0.74	4.50
AMI	1.17	1.52	1.21	1.66	1.07	1.36	1.20	1.50

In both locations, the mean connectance *C* in a bare sand food web (BMSM: 0.068 ± 0.006; Boulogne: 0.099 ± 0.026) exceeds the mean *C* in a reef food web (BMSM: 0.039 ± 0.012; Boulogne: 0.039 ± 0.005). Dividing *T..* by the number of links (*L*) gives the average link weight ($\overline{T}_{ij}$), which is considerably higher in a reef food web compared to a bare sand food web but it is less high for the BMSM (reef: 2.31 ± 0.98, bare sand: 0.44 ± 0.10) compared to Boulogne (reef: 4.73 ± 0.23, bare sand: 0.62 ± 0.11). The mean *AMI* is higher in a reef food web versus a bare sand food web, both in the BMSM (reef: 1.59 ± 0.07, bare sand: 1.19 ± 0.02) and Boulogne (reef: 1.43 ± 0.07, bare sand: 1.13 ± 0.06).

Discussion

Biogenic reefs created by *Lanice conchilega* are prominent habitats in soft-bottom intertidal areas. Recent studies have produced comprehensive data on its physical and biological characteristics (*e.g.* Rabaut *et al.* 2009 for an overview), but studies related to the associated reef food web are scarce and non-quantitative (De Smet *et al.* in press). This study provides the first quantification of the carbon flows in a food web dominated by an ecosystem-engineered *L. conchilega* reef and in an adjacent site without a reef. Network analysis facilitated the comparison of the complex food web interactions, so

that the influence of the specific characteristics of a *L. conchilega* reef on the structure and functioning of a soft-bottom intertidal food web could be identified.

Data quality and the food web model

Most studies on inverse models make use of conventional size-based compartmentalization and feeding types to define the food web compartments. Additionally, many large and diverse groups (*e.g.* fishes) tend to be lumped in one box, masking any detail about their specific interactions. The uniqueness of this study lies in its exceptionally high resolution: 48 single-species compartments were included in the model (Fig. 3 and 4). The species compartments were selected out of all species occurring in the areas based on their abundance and biomass in the system (De Smet *et al.* 2015). Due to a lack of data on meiofaunal and detrital compartments, the focus of this model lies on the macrofaunal part of the *L. conchilega* reef and bare sand food webs. The developed food web model used a combination of site-specific biomass and stable isotope data, and empirical data and physiological constraints from the literature. Most of the eight food web models could not be solved when the input to the sediment comprised only the *in situ* primary production, as derived from measured phytoplankton and microphytobenthos stocks. The site-specific measurements of the phytoplankton stocks and production are expressed per surface area, without taking into account the continuous water flow over the sampled plots. In order to complement the insufficient POC input, an additional input of carbon was added to the model (external phytoplankton, [DETRIT]), and its value left to be determined in the model solution; this will be discussed below. The microphytobenthos stock was estimated from the Chl *a* concentration of the upper sediment layer and is therefore most probably a mixture of benthic diatoms and primary producers from the water column. Hence, the *in situ* production of microphytobenthos can be considered a maximum value.

Carbon flows in *Lanice conchilega* reef areas

The total input of carbon required to sustain the *L. conchilega* reef was high and equalled about 191 ± 50 mmol C m^{-2} d^{-1}, whereas the *in situ* production was about 10 and 40 mmol C m^{-2} d^{-1} in phytoplankton and microphytobenthos respectively. This

indicates that *L. conchilega* and its associated macrofaunal community fulfil their carbon requirement by filtering from a much larger area than the overlying water column. In order to bridge the gap, 1 m^2 of *L. conchilega* reef taps its energy from at least 15 m^2 of water. The combined input of *in situ* produced and laterally advected external phytoplankton fuels most of the carbon input to the reef (80 ± 3% of the total C input), supporting the high biomass of suspension feeders (83 ± 6% of the total biomass in a reef) such as the bivalve *C. edule* (24 ± 14 % of the total biomass) and *L. conchilega* itself (59 ± 14 % of the total biomass). Although *L. conchilega* is a surface deposit feeder, this species has the ability to switch to suspension feeding in case the amount of food on the bottom is limited (Buhr 1976; Fauchald and Jumars 1979). This is particularly advantageous to avoid competition in densely populated reef habitats (Buhr and Winter 1977), such as the reefs modelled in this study. The development of different particle capture and selection strategies reduces the competition between suspension feeders (Lefebvre *et al.* 2009) and might explain the coexistence of high abundances of *L. conchilega* and the suspension feeding bivalve *C. edule* in the reef of the BMSM.

The increased bentho-pelagic coupling in the presence of *L. conchilega* can be attributed to the trapping function of the reef habitat created by the tube-building polychaete (Fig. 5). Dense *L. conchilega* aggregations facilitate the deposition of particulate matter by changing the hydrodynamics on small geographical scales (Friedrichs *et al.* 2000; Rabaut *et al.* 2007a; Borsje *et al.* 2014), more specifically by decreasing the flow velocity at the sediment-water interface via a so-called skimming flow (Luckenbach 1986). Model results show that for low population densities, the flow velocities within a *L. conchilega* patch are strongly reduced and hence particle deposition was enhanced (Borsje *et al.* 2014). Via a positive feedback loop, the effects caused by low density reefs may result in an increasing population density, which on its turn enhances the particle deposition (Borsje *et al.* 2014). Particle deposition was however shown to be limited for population densities exceeding 3000 ind.m^{-2} (Borsje *et al.* 2014). An increased food availability was already assumed for subtidal reef habitats (Van Hoey *et al.* 2008) and is now shown to also hold for the intertidal. Similar focussing of organic matter was for instance reported for subtidal reef structures created by the cold-water coral *Lophelia pertusa* at 800 m depth at Rockall Bank (van Oevelen *et al.*

2009), and for mixed blue mussel (*Mytilus edulis*) and Pacific oyster (*Crassostrea gigas*) reefs in the intertidal flats of the Dutch Wadden Sea (van der Zee *et al.* 2012). In these bivalve reefs, the distribution of several macrobenthic species and shorebirds is strongly affected by the increase in organic matter; demonstrating that the bivalve reefs can affect consumer-resource interactions far beyond their own physical spatial boundaries in intertidal soft-sediment systems (van der Zee *et al.* 2012).

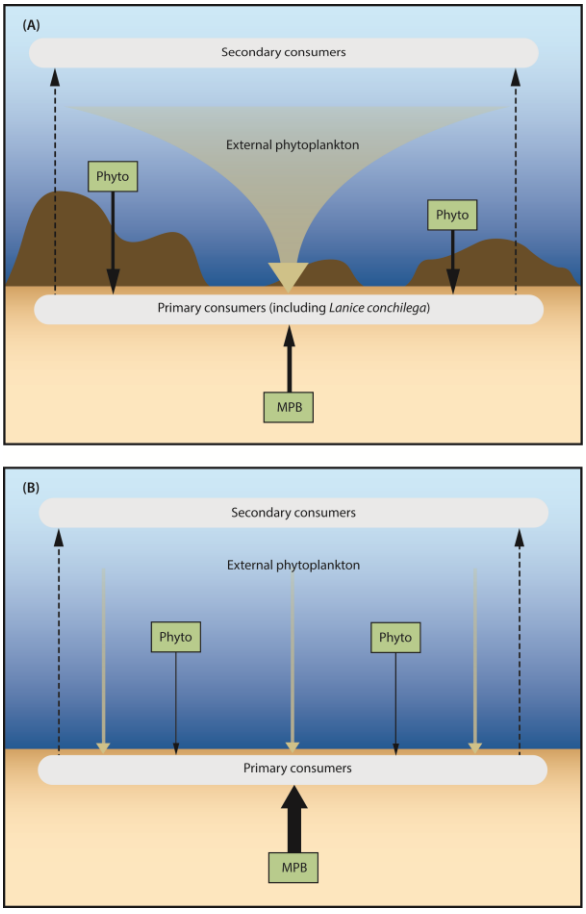

Figure 5. Schematic representation of the important driving role of Lanice conchilega *in the carbon flows (arrows) and dynamics in a soft-bottom intertidal food web. In the presence of the tubeworm (A) the water column-derived input of carbon (= in situ produced phytoplankton (Phyto) + external phytoplankton) prevails due to the trapping of organic matter by the reef structures. In a bare sand area (B) the carbon throughput is much lower and mainly dominated by the input of carbon from in situ primary production by MPB (microphytobenthos). Secondary consumers represent all organisms in the model which do not feed on phytoplankton or microphytobenthos. Arrow thickness represents the magnitude of the carbon flows*

Additionally, van der Zee *et al.* (2012) show that the presence of *L. conchilega* in the bio-engineered bivalve reefs locally enhances the engineering effect of the reefs on the benthic and shorebird community. In the present study, the intertidal *L. conchilega* reefs are similar to the bivalve reefs with respect to their large-scale engineering affects. The facilitated organic matter trapping by the reefs and the consequential impacts on the macrobenthic community and the fish and wading bird populations (De Smet *et al.* 2013; De Smet *et al.* 2015) demonstrate that the ecological impact of *L. conchilega* reefs is much larger than their size may suggest.

Notwithstanding the important carbon flow from primary food sources to *L. conchilega*, the direct carbon throughput from the tubeworm to other macrofaunal compartments in the reef equalled zero. This finding opposes observations showing that *L. conchilega* is a prey item for higher trophic levels such as (flat)fishes (Amara *et al.* 2001) and waders (Goss-Custard and Jones 1976; Yates *et al.* 1993; De Smet *et al.* 2013), and therefore, the flows from the tubeworm to other organisms are probably underestimated by the model. Nevertheless, the model outcome gives a good indication of the relatively limited role of *L. conchilega* in direct trophic interactions within the reef. Overall, the tubeworm creates the environmental conditions to sustain the food web, but itself represents a sink of carbon rather than an important conduit to sustain other macrofaunal organisms. The large biomass of suspension feeders not available for consumption within the system was previously assumed to explain the low transfer efficiency from primary producers to higher trophic levels in the BMSM (Leloup *et al.* 2008).

Carbon flows in bare sand areas

In order to match the macrofaunal biomass present in 1 m^2 of a *L. conchilega* reef, 105 m^2 of bare sand would be required. Lower biomass in these bare sand areas results from the total carbon input which is two orders of magnitude lower (5 ± 2 mmol C m^{-2} d^{-1}) than in the reef areas. Given the production in the overlying water column, this suffices to sustain the macrofaunal community. Moreover, only a small fraction (8.2 ± 4%) of the *in situ* produced primary production in bare sand areas was consumed by the macrofaunal community. Also, the partitioning of the carbon input from the primary food sources to

the macrofaunal community differs considerably in the bare sand areas. In comparison to the *L. conchilega* reef areas, the water column-derived fraction of the carbon input in bare sand areas amounts only 42 ± 13%, which is about half the amount of the water column-derived carbon input in the reef. In bare sand areas, the main supply of carbon is rather provided by the *in situ* production of microphytobenthos (58 ± 13%) (Fig. 5).

Network characteristics

Network indices are convenient to represent certain aspects of food webs (Kones *et al.* 2009) and to facilitate comparison amongst them. Although it is recommended to calculate network indices on the median values from the likelihood approach (Kones *et al.* 2009), in this study, this was not feasible due to the large number of compartments in the inverse food web models. Whereas the use of the parsimony approach in this study may underestimate the network indices of the food webs (Kones *et al.* 2009), as all webs were based on the same overall topological food web with the same food web flows and units, the calculation of the network indices is consistent, justifying the comparison of the presented network indices between the food webs (Kones *et al.* 2009).

The high carbon flow in the reef food webs is reflected in the total system throughput index (*T..*), which sums carbon flows to obtain a measure of the total food web activity (Ulanowicz 2004) and which is 3 to 17 times higher in reef ecosystems. The presence of *L. conchilega* not only increases the overall activity, it also entails a more diverse macrofaunal community, with 10 to 80% more compartments being present in the reefs. This is also reflected in the complexity of the food webs which can best be appraised by comparing the on- and off-reef webs in Fig. 3 and 4, showing much more sparsely connected food webs in bare sand sites. The average mutual information index (*AMI*), which assesses the developmental status of an ecosystem and quantifies how orderly and coherently food web flows are connected is indeed highest for reef areas, which is consistent with the stabilising effect of the ecosystem engineering activities of *L. conchilega* (Godet *et al.* 2009; Rabaut *et al.* 2009).

Location differences

Although the overall differences between on- and off- reef food webs are qualitatively similar, there are also differences in the intertidal food webs in the two study locations. Reefs in Boulogne had a much higher *L. conchilega* biomass and therefore also higher total carbon flows (Table 5). Another consequence of the higher tubeworm biomass is the elevated carbon ingestion and respiration in the reef food web of Boulogne; despite the complete absence of the cockle *C. edule* in the sampling areas of Boulogne. Differences in the *L. conchilega* density between both locations was previously identified as the main driver of differences in the macro- and epifaunal community descriptors (*e.g.* species abundance and diversity) between reef and bare sand areas; being more pronounced in Boulogne (De Smet *et al.* 2015).

Conclusion

The influence of large polychaete populations in shallow coastal areas is often underestimated and their ecological role in the coastal food web is only poorly known (Dubois *et al.* 2009). The use of linear inverse modelling in this study provides new insights in the functioning of a *L. conchilega* reef and its role in a soft-bottom intertidal food web. We show that the presence of biogenic reefs constructed by the ecosystem engineer *L. conchilega* focusses OM produced in an area at least one order of magnitude larger than the reef itself. The tubeworm thus reinforces the bentho-pelagic coupling by establishing reef structures which act as a trap of organic matter. As this also benefits other organisms, the resulting food web in the presence of the tubeworm is much more diverse. However, the worm has only a facilitating role; it does not constitute a significant source of carbon for other macrofaunal organisms. The effect of *L. conchilega* on the carbon dynamics in a reef food web seems to be applicable to locations with different environmental conditions, but can differ in magnitude depending on the density of the tubeworm reefs.

Acknowledgements

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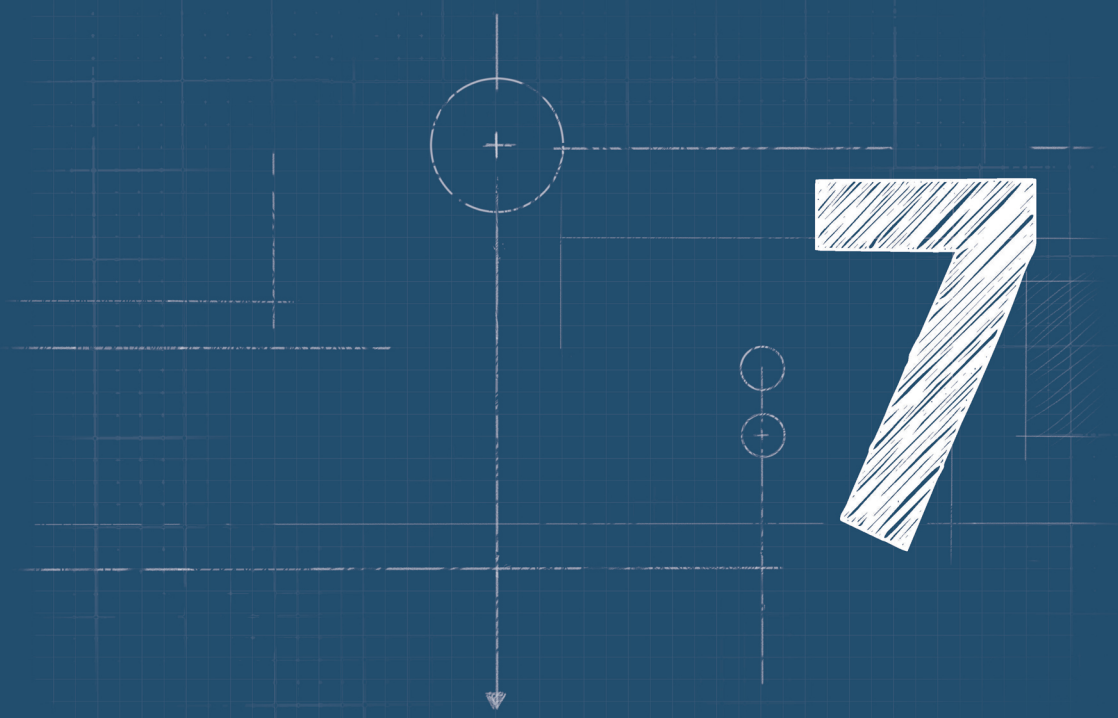


Chapter 7

General discussion, conclusions and future challenges

A broad range of studies has investigated the effect of ecosystem engineers on biodiversity and ecosystem functioning in various ecosystems (*cfr.* Chapter 1). The outcome of these studies makes ecosystem engineers particularly useful conservation targets (Crain and Bertness 2006). In order to increase the value of the engineering concept in ecology and conservation biology, research on the critical role of ecosystem engineers in the structure and function of ecosystems should carry on (Crain and Bertness 2006; Braeckman *et al.* 2014). However, rather than examining ecosystem engineer effects on small, fragmented parts of the ecosystem, studies focussing on the effect of ecosystem engineers on entire communities and food webs are required. This PhD thesis is aimed at improving our understanding of the structural and functional role of biogenic reefs constructed by the ecosystem engineer *Lanice conchilega* in natural soft-substrate coastal ecosystems. This was achieved by first studying the effect of *L. conchilega* reefs on the bentho-pelagic community (Chapter 2) and the wading bird community (Chapter 3) associated with the reef habitats. Secondly, predator effects on functional aspects of the ecosystem engineer were investigated (Chapter 4). Thirdly, the trophic interactions taking place within the reef habitats were characterised and research on ecosystem engineering and trophic interactions (*i.e.* food web ecology) was comprehensively combined (Chapter 5). Finally, the carbon flows of a soft-bottom intertidal food web in the presence of ecosystem-engineered *L. conchilega* reefs were quantified (Chapter 6). By comparing the ecosystem in the presence and absence of *L. conchilega* in Chapters 2, 5 and 6, the overall added value of a *L. conchilega* reef to the functioning of the ecosystem could be explored.

This chapter combines the results of this thesis and discusses the position of *L. conchilega* reefs with respect to the community composition, feedback effects, food web structure and carbon cycling in intertidal areas. This PhD thesis integrates the knowledge on the effects of *L. conchilega* reefs on the intertidal ecosystem level and beyond the local scale. The added value of *L. conchilega* reefs in otherwise uniform intertidal areas is revolved around the conceptual diagram introduced in Chapter 1, providing an overview of the potential interactions involving the ecosystem engineer *L. conchilega* (Fig. 1).

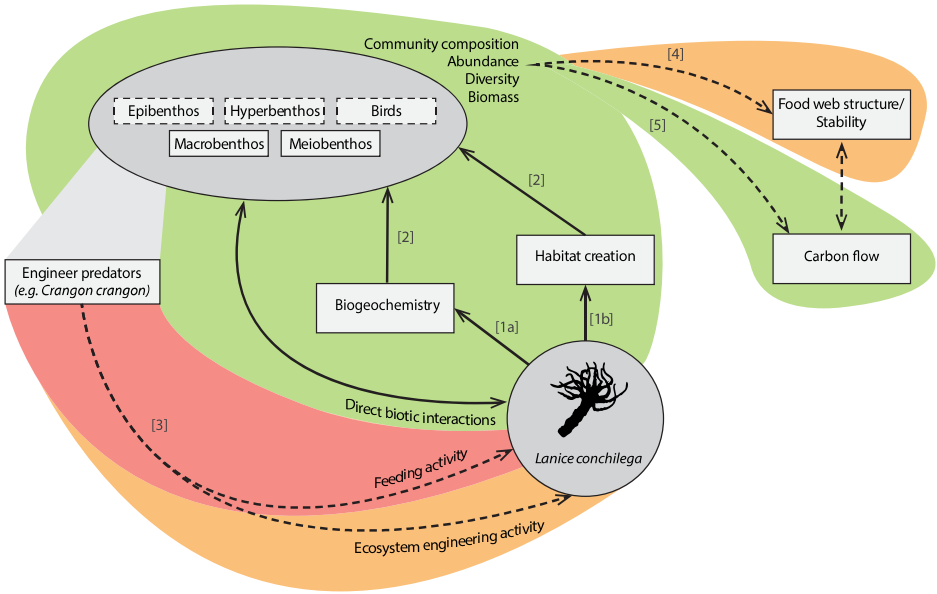


Figure 1. Schematic overview of the potential interactions involving the ecosystem engineer Lanice conchilega *as introduced in Chapter 1. Dashed boxes and lines represent largely unknown and understudied compartments and links which were dealt with in this PhD thesis and which are integrated in this Chapter. Colours indicate whether the compartments/links were found to be positively (green), negatively (red) or not (orange) affected. [x] refers to the investigated interactions discussed in the paragraphs of this chapter*

The added ecological value of intertidal *Lanice conchilega* reefs

Ecosystem engineers are regarded critical species in determining the ecosystem integrity and functioning. Therefore, the identification and preservation of ecosystem engineering species and responsive ecosystems is pushed forward for conservation

(Byers *et al.* 2006). As some engineering species have large-scale effects on abiotic conditions, their introduction or loss could drastically alter the abiotic system state, triggering a consequent response in the biotic state (Byers *et al.* 2006). Kauppi *et al.* (2015) illustrated such drastic alterations by investigating the introduction of the invasive and bioirrigating polychaete *Marenzelleria spp.* in the Baltic Sea (*see* Chapter 1). The strength of the effect on the local biotic and abiotic properties of the environment will however depend on the engineering capacities of the species, as well as on the spatial and temporal sustainability of the engineer population. The polychaete *Lanice conchilega* is an excellent example of an engineering species shown to be useful in a nature conservation policy context. Moreover, *L. conchilega* is labelled as a reef building species for which a conservation framework is at hand (Braeckman *et al.* 2014). *Lanice conchilega* is bound to receive a higher ecosystem engineer qualification compared to other species due to its combined allogenic and autogenic engineering capacities (Braeckman *et al.* 2014). The tubeworm's engineering activities affect the biogeochemical characteristics (Forster and Graf 1995; Braeckman *et al.* 2010) and alter the physical structure of the abiotic environment (*e.g.* Degraer *et al.* 2008; Rabaut *et al.* 2009) (Interaction [1a] and [1b] in Fig. 1). As a result, the reefs affect the composition, species richness, abundance and biomass of the associated faunal communities (Fig. 1, [2]). *Lanice conchilega* reefs thus have a strong structural role, which even persists in the presence of high abundances of predators feeding in the reefs (Fig. 1, [3]). Despite this structuring role, neither food web structure nor food web stability is altered by the reefs (Fig. 1, [4]), however the magnitude of the links between different food web compartments is modified by the increased carbon input in the presence of the reefs (Fig. 1, [5]).

Lanice conchilega reefs modify their environment

Bioirrigation

One of the most striking engineering activities affecting the biogeochemical characteristics of *L. conchilega* reefs is the process of bioirrigation (Forster and Graf 1995; Braeckman *et al.* 2010) (Fig. 1, [1a]). Bioirrigation *sensu stricto* is the enhanced exchange

between the pore water and the overlying water column owing to burrow flushing (Meysman *et al.* 2006). Due to its tube-dwelling lifestyle, *L. conchilega* ventilates its tube by so-called piston-pumping, *i.e.* water is drawn into the tube and pushed out whenever the worm moves within its tube (Forster and Graf 1995). By means of this activity, the tube-building polychaete advects oxygenated water into the sediment surrounding its tube (Forster and Graf 1995), leading to an increased benthic respiration, nutrient release and denitrification (Braeckman *et al.* 2010). However, so far only microcosm and single-species approaches were used to demonstrate the bioirrigation capacity of *Lanice conchilega*. In this thesis, a *L. conchilega* reef approach (with tubeworm densities up to 4000 ind.m^{-2}) was adopted, which more faithfully reflects the natural situation (Chapter 4). The bioirrigation rate of a *L. conchilega* reef was found to equal about 30 litre m^{-2} d^{-1}, which is more than twice as high as previously reported bioirrigation rates of *L. conchilega* (Forster and Graf 1995). Compared to other bioirrigating macrofauna such as the burrowing lugworm *Arenicola marina* (120-160 litre m^{-2} d^{-1}) (Kristensen 2001) and the burrowing shrimp *Trypaea australiensis* (429 litre m^{-2} d^{-1}) (Webb and Eyre 2004), the irrigation rate of the *L. conchilega* reef is rather low. In contrast to faunal burrows, the physical barrier created by the *L. conchilega* tube is expected to slow down the exchange of water between the inner tube and the surrounding sediment. Nevertheless, the high tubeworm densities and the resulting increase in bioirrigation in a reef habitat is assumed to increase the oxygen penetration depth in the sediment surrounding the tubes (Braeckman *et al.* 2010). The oxygenation of the sediment in combination with for example a stimulation of the microbial community in the sediment surrounding the tubes (Solan and Wigham 2005) positively affects meiofaunal communities associated with the reefs (Zühlke *et al.* 1998; Braeckman *et al.* 2011b). The bioirrigation activity in a *L. conchilega* reef as seen in Chapter 4 thus has a considerable share in explaining the meiofaunal community composition, abundances and diversity, and might even affect some macrofaunal species such as the amphipod *Urothoe poseidonis* (Callaway 2006).

The increased bentho-pelagic coupling in intertidal areas

Lanice conchilega is known to improve the quality of subtidal habitats by creating dense reef structures which reduce the water flow velocity and subsequently increase the food

availability in the habitat (Van Hoey *et al.* 2008) (Fig.1, [1b]). The changes in the hydrodynamic regime consist of a decrease of flow velocity at the sediment-water interface (a so-called skimming flow; Luckenbach 1986). The increased food availability in the subtidal area is now also shown for intertidal areas (Chapter 6). In the intertidal zone, dense tubeworm aggregations locally increase the bentho-pelagic coupling by acting as traps of organic matter from the water column, supporting the overall high macrofaunal biomass. In other words, the reefs and their associated macrofauna largely depend on the input of carbon from the water column rather than from *in situ* primary production.

Lanice conchilega reefs structure the associated faunal communities

The macrobenthic and meiofaunal communities (mainly studies on nematodes) associated with *L. conchilega* reefs have been repeatedly shown to benefit from the ecosystem engineering activities of *L. conchilega* (*e.g.* Zühlke *et al.* 1998; Rabaut *et al.* 2007a; Van Hoey *et al.* 2008; Braeckman *et al.* 2011b). Similarly, the scarce and non-integrated studies on flatfishes and birds within the *L. conchilega* reef habitats reveal a positive bottom-up link from the reefs to these organisms (*e.g.* Godet *et al.* 2008; Rabaut *et al.* 2013). Whereas the effect of *L. conchilega* reefs on the associated macrofauna and birds is studied in isolation, this thesis is the first study including all the ecological groups present in the vicinity of the reefs and investigating them with a high resolution. Our elaborate and integrated sampling effort confirmed hitherto assumed simultaneous effects of *L. conchilega* reefs on the macro-, epi-, and hyperbenthic communities (Chapter 2) and the community of wading birds (Chapter 3) (Fig.1, [2]). The macrobenthos was observed to be mainly structured by the high tubeworm densities, and the resulting increase in habitat heterogeneity and shelter/refuge provision. Until now, flatfish were assumed to be attracted to the reefs because of two reasons: (1) the provision of shelter and/or (2) the availability of food (Rabaut *et al.* 2010; Rabaut *et al.* 2013). The present study showed that flatfishes (and epifauna in general) are mostly attracted to the reefs by the increased macrobenthic diversity and abundance, hence by the availability of food rather than by the provision of shelter. Their high mobility enables the epibenthos to actively move to and feed upon the macrobenthos within the

reef habitat. Similarly, the hyperbenthos (small animals living in the water layer close to the seabed; Mees and Jones 1997) was shown to be structured by the presence of *L. conchilega*, however the structuring effect was less strong compared to the macro- and epibenthos. The lesser dependence of hyperbenthic animals to the seabed, and hence to the biogeochemical and physical changes of the sediment resulting from the engineering activity of *L. conchilega*, is assumed to explain their rather limited reef selectivity. The increased wader densities in the *L. conchilega* reef of the Bay of the Mont Saint-Michel (wader densities in the reef exceeded these in bare sand areas about 47 times; Chapter 3) can be related to the reefs' elevated food supply, *i.e.* the high abundance and biomass of associated macro-, epi-, and hyperbenthic organisms. Indeed, faecal analysis showed that birds in the reefs preferentially feed on the fauna associated with the tubeworm (Chapter 3). Especially crustaceans are noted to be important in the diet of waders feeding in the reef, while the share of *L. conchilega* itself in their diet is rather limited.

The large amounts of predators attracted to the *L. conchilega* reefs are assumed to increase the predation pressure on the associated fauna and the tubeworm itself (Chapter 4) (Fig.1, [3]). Indeed, the feeding activity of *L. conchilega* in the continuous presence of a predator was shown to be reduced to a sub-optimal level. The engineering activity of *L. conchilega* however was demonstrated to remain unaffected in the presence of the predatory shrimp *C. crangon*. As such, the polychaete can maintain its important structural and functional role within soft-bottom intertidal areas, even under high predatory pressure.

In general, the effect of *L. conchilega* reefs on the associated fauna studied in this thesis confirms the structural role of the reefs, and provides insights in previously under- or entirely unstudied faunal compartments such as the epi-, and hyperbenthos. Overall, these new findings complement and reinforce the existing knowledge on the central role that *L. conchilega* reefs play in linking benthic, pelagic and air-borne compartments of soft-bottom intertidal food webs.

The role of *Lanice conchilega* reefs relative to other biogenic habitats

The structuring and functional role of *L. conchilega* reefs was found to have far reaching implications for the macro-, epi- and hyperbenthos and the wader community associated to the reefs. Next to *L. conchilega* reefs, several other examples exist of biogenic structures which are relevant to temperate coastal ecosystem functioning (*e.g.* bivalve aggregations, maerl beds and seagrass beds). A brief and non-exhaustive overview of important biogenic structures in temperate areas is given below. Additionally, in the light of this discussion, we provide a first attempt to compare the effects of a variety of ecosystem engineers on physical, biological and socio-economic aspects of the ecosystem they are part of.

Besides *L. conchilega*, other reef-building polychaetes belong to the family Sabellariidae, including the characteristic species *Sabellaria alveolata* in temperate areas (Holt *et al.* 1998; Dubois *et al.* 2002; Dubois *et al.* 2006). As for *L. conchilega*, large patchy reefs of this tubeworm are present in the intertidal area of the Bay of the Mont Saint-Michel, covering approximately 100 ha (Dubois *et al.* 2002). The large (up to 60 cm) and solid reef structures change the physical environment (Holt *et al.* 1998) and consequently add high levels of biodiversity to the otherwise low biodiversity soft-bottom intertidal areas of the BMSM (Dubois *et al.* 2002). Both for *L. conchilega* and *S. alveolata* reefs, the unique nature of the community found on the reefs is not related to the presence of a particular species, but is rather due to the juxtaposition of species belonging to the surrounding communities (*i.e.* the so-called 'Babushka-like pattern'; Dubois *et al.* 2002; Rabaut *et al.* 2007a; Van Hoey *et al.* 2008) (Table 1). Moreover, similarly to *L. conchilega* reefs, *Sabellaria* reefs can appear and disappear rapidly, but they do have similar mechanisms to enhance stability and longevity too (*i.e.* strong recruitment, sediment stabilisation and consolidation) (Holt *et al.* (1998) and references therein). In general, the influence of large *Sabellaria* spp. populations in shallow coastal areas and their ecological role in the coastal food web remains until now poorly understood (Dubois *et al.* 2009) (Table 1).

The blue mussel (*Mytilus edulis*) and the Pacific oyster (*Crassostrea gigas*) are two important reef-building bivalve species reaching from the intertidal to the shallow subtidal zone. Bivalve aggregations create hard substrata and introduce complexity and

heterogeneity into benthic environments and as such are important elements of habitat structure affecting population-, community- and ecosystem-level processes (Guttiérez *et al.* (2003) and references therein). Similar to polychaete reefs, reefs constructed by bivalves can positively affect the species richness by providing substrata for attachment, forming a refuge for different mobile organisms or providing resources by reducing the hydrodynamics (Gutiérrez *et al.* 2003). In analogy with *L. conchilega* reefs, reef-building bivalves importantly affect the local benthic community (Norling and Kautsky 2008) and they constitute important feeding grounds for birds (Nehls *et al.* 1997; Caldow *et al.* 2003) (Table 1). Moreover, in soft-bottom intertidal areas, bivalve reefs can affect consumer-resource interactions far beyond their own physical spatial boundaries, meaning that the ecological impact of the reefs is much larger than their actual size suggests (van der Zee *et al.* 2012). Additionally, van der Zee *et al.* (2012) show that the presence of *L. conchilega* in the biogenic bivalve reefs locally enhances the engineering effect of the reefs on the benthic and shorebird community. Whereas intertidal *L. conchilega* reefs were shown to have large-scale engineering effects (Chapter 6) and consequently impact the macrobenthic, fish and wading bird communities (Chapters 2 and 5), they can be regarded to have a far reaching ecological role similar to the role of bivalve aggregations.

Recently, maerl beds, carbonate deposits characterised by accumulations of unattached calcareous red algae (Corallinales, Rhodophyta), have been recognised as important on a European scale because their complex architecture and extreme longevity account for a high biodiversity and provide essential areas for commercial fishing (Hall-Spencer *et al.* 2003; Wilson *et al.* 2004). Maerl beds occur in coastal areas worldwide and appear as poorly sorted, patchy and complex habitats that greatly differ in their structure from more uniform bare sand or mud habitats (Grall *et al.* 2006). Maerl beds harbour a high local biodiversity compared to other sedimentary habitats (Steller *et al.* 2003; Jackson *et al.* 2004) and often provide protection for commercially important species (Steller *et al.* 2003; Kamenos *et al.* 2004; Nelson 2009). Both maerl beds and *L. conchilega* reefs locally increase the microphytobenthos biomass and trap phytoplankton, but maerl beds provide an additional primary food source being macroalgae (that can be divided between live maerl fragments and epiphytic soft macroalgae) (Grall *et al.* 2006). The

diversity of primary food sources combined with the physical complexity due to the accumulation of maerl fragments is assumed to give rise to a wide variety of microhabitats that could explain the high macrobenthic species richness and biomass observed in these habitats (Grall *et al.* 2006). The structural and functional ecology of the peculiar and complex maerl habitats has however received very little attention in contrast to other marine communities such as kelps forests or seagrass beds.

Macroalgae, and in particular the kelp *Laminaria hyperborea*, have been shown to house a large and diverse community of invertebrates (*e.g.* Moore 1973a; Moore 1973b). The brown algae *L. hyperborea* covers extensive areas and is widely distributed in the north-east Atlantic (Kain 1967). Kelp can be divided in three parts (frond, the stipe and the holdfast or hapteron) and provides a large and heterogeneous habitat because of its height (up to 12 m) and shape. The holdfast is the most complex structure of the kelp and consequently harbours the most abundant and diverse meio- and macrofauna (Norderhaug *et al.* 2002; Christie *et al.* 2003; Arroyo *et al.* 2004) compared to the remaining parts of the kelp or adjacent bare sand habitats. In general, kelp forest systems produce large numbers of potential prey organisms for predators such as fish, crabs, lobsters and seabirds (Norderhaug *et al.* 2002; Christie *et al.* 2003).

Seagrass beds are among the most important marine habitats in the world and they can cover large areas of soft sediments in shallow coastal waters. While individual seagrass shoots have a life span of weeks or decades, seagrass beds can in extreme cases persist for centuries or millennia (Hemminga and Duarte 2000). Seagrass beds are highly productive systems (Duarte and Chiscano 1999) and provide a high level of physical structure (up to 6 m; Aioi *et al.* 1998), food (especially organic detritus) and shelter for a wide range of organisms. Consequently, seagrass beds support richer and more diverse associated macrofaunal communities compared to the surrounding bare sand areas (Edgar *et al.* 1994; Connolly 1997; Lee and Fong 2001). Moreover, rather than extending the soft-bottom macrofaunal community (as is the case for *L. conchilega* reefs), seagrass beds harbour species which are not found in merely soft sediments (Lee and Fong 2001).

Reefs created by cold-water corals are widely distributed ecosystems along the continental margins. Cold-water corals thrive in dark, cold and mostly deep oceanic waters and they occur in a variety of growth forms, ranging from individuals up to giant carbonate mounds (up to 300 m high; Roberts *et al.* 2006). Coral communities seem to be fuelled by primary production in the surface waters and the subsequent downslope transport of this organic-rich surface water to the sea floor (Duineveld *et al.* 2004; Duineveld *et al.* 2007). The increased concentration of organic matter was shown to result from the ecosystem engineering properties of reef structures created by the cold-water coral *Lophelia pertusa* (van Oevelen *et al.* 2009) and is comparable to the trapping function of *L. conchilega* reefs as demonstrated in this thesis. Due to their high structural complexity, cold-water corals provide niches for many species (Roberts *et al.* 2006) and support a highly diverse fauna similar to those found in tropical shallow-water reefs (*e.g.* Jensen and Frederiksen 1992). However, until now, little knowledge on the functional relationships between species on cold-water corals exists (Roberts *et al.* 2006).

The physical and biological impacts of a *L. conchilega* reef on its environment, as well as its role in coastal protection, are compared to other ecosystem engineered habitats and summarised in Table 1. Based on this first evaluation, the impact of *L. conchilega* reefs seems rather limited, which can be attributed to the small to moderate height and the ephemeral character of most intertidal *L. conchilega* reef formations (Table 1). Consequently, although *L. conchilega* reefs were shown to largely affect the benthic assemblage, the ecosystem's influence on the water column can be regarded low compared to other habitat building ecosystem engineers such as kelp forests or seagrass beds. Therefore, in locations where *L. conchilega* reefs coexist with other ecosystem engineers (such as *S. alveolata* reefs in the BMSM), the added value of *L. conchilega* is limited. Nonetheless, in coastal systems where the only reef-building ecosystem engineer appears to be *L. conchilega*, the reefs' impact can be assumed to be much more substantial.

Next to impacts on the physical and biological aspects of the environment, we focussed on the ability of ecosystem engineers to contribute to coastal protection via *e.g.* wave attenuation and shore line stabilisation. Wave attenuation can be provided by any

intertidal ecosystem that creates aboveground structures of significant size such as biogenic reefs, seagrass beds and kelp forests (Bouma *et al.* 2014). Seagrass beds for example reduce the hydrodynamics from currents and waves (Gambi *et al.* 1990) and stabilise sediments (Fonseca 1989) resulting in the prevention of sediment erosion. Therefore, extensive seagrass beds have a stabilising function in coastal areas and provide, among other ecological services, a natural form of coastal protection (Barbier *et al.* 2011). The coastal protection capacity of reefs created by oysters, mussels and *Sabellaria* spp. is less studied (but see *e.g.* Borsje *et al.* 2011; Donker *et al.* 2013) and may be less effective since they are usually found below mean sea water level (Bouma *et al.* 2014). Nevertheless, their rigidity and role in sediment stabilisation make biogenic reefs efficient breakwaters (Borsje *et al.* 2011). No information on the importance of biogenic *L. conchilega* reefs for coastal protection is available, however despite their sediment stabilising function, their rather ephemeral character in combination with the "soft" structures created by the tubeworm, may render the coastal defence value of the reefs relatively low compared to other ecosystem engineers in coastal systems.

Notwithstanding the limited ecosystem engineering impacts of *L. conchilega* reefs compared to other autogenic engineers, *L. conchilega* is at the same time an allogenic engineer and hence largely affects the belowground part of the ecosystem. Changes in the sediment biogeochemistry as a result of the tubeworm's bioirrigation activity contribute to changes in the species composition of smaller interstitial fauna (see the discussion on '*Biorrigation*'). The unique combination of autogenic and allogenic engineering effects of the tubeworm, render *L. conchilega* reefs important habitats which affect the environment at a much larger scale than the scale of the individual. Therefore, *L. conchilega* must be considered an important ecosystem engineer that does not need to be protected as a species by itself, but because of its value in ecosystem functioning (Braeckman *et al.* 2014).

Table 1. Overview of important biogenic structures in temperate areas and their impact on physical (elevation, longevity, organic matter trapping), biological (biodiversity, food web effect and allogenic ecosystem engineering effect) and socio-economic aspects (coastal protection) of the ecosystem they belong to. The color code reflects a classification from a low (light grey) to a high (black) impact on the ecosystem; enabling an enhanced comparison between biogenic structures.

	Elevation	Longevity	Biodiversity	Food web effect	Trapping of OM	Allogenic effect	Coastal protection
***L. conchilega* reefs**	0.075 - 0.8 m [1,2,3]	Ephemeral [6,7]	Babushka effect [4,5]	Yes/No [0]	Yes [0]	Yes [0,8,9]	Low
***S. alveolata* reefs**	up to 0.6 m [6]	Moderate life-span [6]	Babushka effect [10]	N/A	Yes [28]	No	Moderate [31]
Bivalve reefs	0.3 - 1.2 m [6]	Moderate life-span [11]	Moderate increase [6,17]	N/A	Yes [29]	No	Moderate [30]
Maerl beds	up to 10 m [12]	Extreme longevity [18]	Increase [16]	Yes [37]	Yes [37]	No	N/A
Kelp forests	< 5 - 45 m [13]	Long life-span [19]	Increase [20]	Yes [39]	No [40]	No	Moderate [34, 35]
Seagrass beds	0.5 - 6.8 m [14]	Long life-span [27]	Unique fauna [21,22,23]	Yes [33]/No [32]	Yes [38]	No	High [24]
Cold-water corals	up to 300 m [15]	Extreme longevity [15]	Increase [25]	Yes [36]	Yes [26]	No	N/A

(0) This study, (1) Rabaut *et al.* (2009), (2) Carey (1983), (3) Degraer *et al.* (2008), (4) Rabaut *et al.* (2007a), (5) Van Hoey *et al.* (2008), (6) Holt *et al.* (1998), (7) Callaway *et al.* (2010), (8) Forster and Graf (1995), (9) Braeckman *et al.* (2010), (10) Dubois *et al.* (2002), (11) Zühlke (2001), (12) Grall and Hall-Spencer (2003), (13) Abbott and Hollenberg (1976), (14) Aioi *et al.* (1998), (15) Roberts *et al.* (2006), (16) Steller *et al.* (2003), (17) Norling and Kautsky (2008), (18) Hall-Spencer *et al.* (2003), (19) Dayton *et al.* (1992), (20) Christie *et al.* (2003), (21) Edgar *et al.* (1994), (22) Connolly (1997), (23) Lee and Fong (2001), (24) Barbier *et al.* (2011), (25) Jensen and Frederiksen (1992), (26) van Oevelen *et al.* (2009), (27) Hemminga and Duarte (2000), (28) Desroy *et al.* (2011), (29) van der Zee *et al.* (2012), (30) Borsje *et al.* (2011), (31) Bouma *et al.* (2014), (32) Baeta *et al.* (2009), (33) van der Zee *et al.* (2014), (34) Elwany *et al.* (1995), (35) Mork (1996), (36) Duineveld *et al.* (2007), (37) Grall *et al.* (2006), (38) Gacia *et al.* (1999), (39) Fredriksen (2003), (40) Eckman *et al.* (1989). N/A = no information available.

Towards the integration of ecosystem engineering and food webs

From conceptual theories to empirical evidence

The incorporation of non-trophic interactions, such as ecosystem engineering, in traditional food web studies is increasing (Olff *et al.* 2009; Kéfi *et al.* 2012), but up until now, the significance of ecosystem engineering for food web structure and dynamics remains largely unknown (Sanders *et al.* 2014). To get a more general understanding of interaction webs in nature, the integration of ecosystem engineering and food webs cannot be longer avoided (Sanders *et al.* 2014). Despite the growing interest in the capacity of ecosystem engineers to modify the structure and dynamics of food webs, most studies dealing with this issue have a theoretical nature and empirical evidence is largely lacking (Arditi *et al.* 2005; Goudard and Loreau 2008; Sanders *et al.* 2014). Empirical studies are needed to assay the validity of the theoretical frameworks and define their weaknesses and shortcomings. This PhD thesis provides one of the first attempts to link food web structure to ecosystem engineering activities in soft-bottom intertidal areas. Some of the conceptual frameworks were empirically tested and the outcomes can now be used to adjust and improve the initial concepts.

Lanice conchilega affects the carbon flow, but not the structure of the food web

Sanders *et al.* (2014) recently presented a conceptual framework to integrate the largely independent research areas of ecosystem engineering and food webs. Following this framework, habitat building ecosystem engineers are expected to have an overall impact on the structure of the food web.

The reef-building capacity of *L. conchilega* in combination with its physical and biogeochemical engineering activities, positively affect the availability of primary food sources at the base of the food web (Chapter 6) and consequently abundances and biomasses of higher trophic levels (Chapters 2 and 3). Therefore, the effect of the tubeworm aggregations was expected to be reflected in the global structure of the food web to which the ecosystem engineer belongs (Fig.1, [4]). Despite the considerable structuring role of *L. conchilega*, we provide evidence that the food web structure is only

to a limited extent affected by the presence of the tubeworm reefs. The structure of a food web is largely determined by the primary food sources at the base of the web, fuelling higher trophic levels. Although *L. conchilega* alters the quantity of the carbon sources at the base of the food web, it does not fundamentally change the diversity of the primary food sources in soft-bottom intertidal areas (*i.e.* microphytobenthos and phytoplankton). Moreover, the structuring effects of *L. conchilega* are mainly reflected in the benthic components of the food web and much less in the water-column derived components (*cfr.* the low structuring effect on the hyperbenthos in comparison to the macro- and epibenthos). Since the intertidal food web is mainly driven by a carbon input from the water-column, the global intertidal food web structure is not fundamentally altered in the presence of *L. conchilega*.

Notwithstanding the unchanged food web structure, the reefs do affect the magnitude of the carbon flow in the intertidal food web (Chapter 6) (Fig.1, [5]). As a result of the trapping function of a *L. conchilega* reef, the carbon input in a reef is about 40 times higher compared to a bare sand area. Hence, the amount of carbon processed within the *L. conchilega* food web largely exceeds the transfer of carbon in the absence of the reefs, and enables the occurrence of high macrofaunal biomass. In general, the elevated carbon flow in the presence of the tubeworm results in much more diverse food webs.

Lanice conchilega reefs and food web stability

Several human actions have been implicated in the loss of biodiversity. However, the impact of human activities on food web structure and stability of food webs remains poorly known. Food web theory provides a useful framework allowing the interpretation of potential effects of environmental change on both food web structure and food web stability (McMeans *et al.* 2013). Since *L. conchilega* reefs largely affect the diversity and abundance of a wide variety of faunal groups, it could be hypothesised that in the presence of the reefs, the stability of the food web was positively affected. This was tested in two ways: (1) by the coupling of fast and slow energy channels in space by mobile top predators; and (2) by making use of the Layman metrics introduced in Chapter 5.

Rooney *et al.* (2006) show that a seemingly common structure, the coupling of fast and slow energy channels in space by mobile top predators, impart stability to food webs. The energy channels are based on different basal resources and differ in their turnover rates and productivity. Fast energy channels (*e.g.* phytoplankton energy channels) tend to have smaller, faster growing populations that have higher biomass turnover rates compared with the slow energy channels (*e.g.* detrital energy channels) (Rooney *et al.* 2008). When graphed on axes of percentage reliance on 1 of 2 carbon sources versus trophic position, a 'hump-shaped' pattern is seen (Fig. 2A), which already has been identified in terrestrial, marine and freshwater food webs ranging from temperate to arctic systems (Rooney *et al.* 2006; Dolson *et al.* 2009; McMeans *et al.* 2013). The left side of the graph represents a slow energy channel, characterised by relatively low biomass turnover rates and low interaction strengths between consumers and resources compared to the fast energy channel on the right (Rooney *et al.* 2006).

An attempt to reconstruct this pattern for the soft-bottom intertidal food webs in the absence and presence of *L. conchilega* was unsuccessful (Fig. 2B). This indicates that despite the elaborate and large-scaled sampling approach used in this thesis, data on the primary food sources and/or consumer species within the sampling areas were lacking or not fully available. Especially isotope values from organisms grazing on microphytobenthos were underrepresented (note the lack of taxa in the left side of Fig. 2B). Many documented organisms feeding on microphytobenthos form part of the meiofauna (metazoan organisms that pass a 1 mm sieve and are retained on a 38 µm sieve; mainly dominated by nematodes and harpacticoid copepods) (Miller *et al.* 1996; Maria *et al.* 2012). Since this smaller-sized benthos plays an important role as a trophic linkage to macrofauna, or as important structural components of the benthic community (Coull 1999), they most probably are the missing link in this study. Moreover, the isotope values of top predators in intertidal areas such as waders and seals could not be included because of practical and ethical issues. Overall, this thesis is assumed to focus mainly on the fast energy channel of the intertidal food web (right side of Fig. 2B), driven by a large carbon input from the water column (Chapter 6). Unfortunately, it lacks data on the slow energy channel (left side of Fig. 2B), which depends on the *in situ* primary production and detrital matter in the sediment.

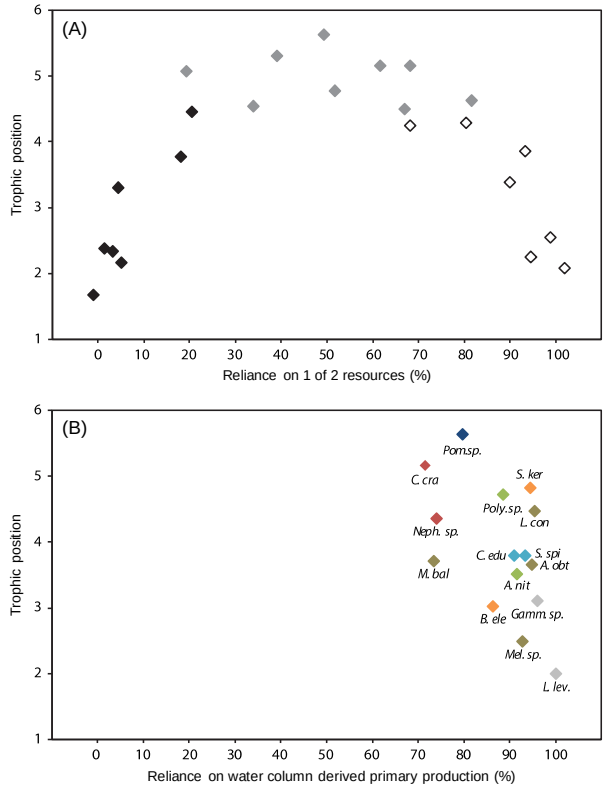


Figure 2. Investigating the link between diversity and food web stability. (A) Conceptual graph showing that food webs are structured such that top predators (grey symbols) couple distinct energy channels (black and white symbols) by preying upon organisms from both channels (adapted from Rooney et al. 2006). (B) Food web representation based on the percentage of carbon derived from primary production in the water column for the L. conchilega *reef of the Bay of the Mont Saint-Michel (BMSM) in spring. Abbreviations: L.mon =* Lekanesphaera levii, *Mel. sp. = Melita sp., B. ele =* Bathyporeia elegans, *Gamm. sp. = Gammarus sp., A. nit. =* Athanas nitescens, *M. bal. =* Macoma balthica, *A. obt. =* Abludomelita obtusata, *S. sp. =* Schistomysis spiritus, *C. edu. =* Cerastoderma edule, *L. conc =* Lanice conchilega, *Neph. sp. = Nephtys sp., Poly. sp. = Polynoinae sp., S. ker. =* Schistomysis kervillei, *C. cra. =* Crangon crangon, *Pomm. sp. = Pomatoschistus sp.*

Although we were unable to investigate the stability of the food web by linking fast and slow energy channels, insights were provided by investigating the density of species packing, and hence trophic redundancy (with an increased trophic redundancy being an indication of many taxa with similar trophic ecologies), from the Layman metrics introduced in Chapter 5. Based on these metrics, we can conclude that in the presence of *L. conchilega* aggregations, not only the structure of the food web, but also its stability remains largely unaltered. Once again, the dependency of the *L. conchilega* reef

community on the input of carbon from the water column rather than on the *in situ* sedimentary primary production most probably explains the unaltered stability.

In order to determine which engineer characteristics are expected to affect food web stability in the absence and presence of extrinsic environmental perturbations, Sanders *et al.* (2014) modified a well-studied three-species food chain model. Their model shows that engineering effects on food web stability might strongly depend on the trophic position of the engineer and complex feedbacks between engineering and trophic effects.

In short, *L. conchilega* can be regarded an ecosystem engineer impacting the nodes of the food web (by affecting the diversity, the abundance (Chapter 2 and 3) and the biomass (Chapter 6) of its associated species (being the nodes)), and hence affecting the magnitude of the links (*i.e.* trophic and non-trophic interactions) between food web compartments (Chapter 6), but not the overall food web structure and stability (Chapter 5).

From *Lanice conchilega* to ecosystem engineers

Only a few recent studies investigated how ecosystem engineering by habitat modifying species affect the structure of the food web in the marine realm (Botto *et al.* 2005; Baeta *et al.* 2009; Rigolet *et al.* 2014b; van der Zee *et al.* 2014), making use of different indicators of food web structure. Van der Zee *et al.* (2014) investigate the effect of the engineering activities of sea grasses and burrowing crabs on the food web structure of the soft-bottom intertidal flats of Banc d'Arguin (Mauritania). They demonstrate that the activities of habitat modifying species can strongly affect food web structure and dynamics by enhancing species numbers on the one hand and changing the linkages and connectance among species on the other hand. The sea grass patches, that accumulate silt and form 3D structures, in combination with the creation of large intertidal pools in the accumulated silt layers by burrowing crabs results in much higher food web impacts compared to the effects of the reef structures built by *L. conchilega*. The impact of *L. conchilega* is largely limited to the sediment, while sea grass meadows reach out in the water column and are assumed to substantially affect the pelagic part

of the food web, which might explain their more pronounced effect on the food web structure in an intertidal area. Nevertheless, this is to our knowledge the only study presenting an effect of an ecosystem engineer on the structure of a food web. Rigolet *et al.* (2014b) studied the effect of the engineering tubicolous amphipod *Haploops nirae* on the benthic food web structure of an *Amphiura filiformis* habitat. Despite an alteration of the local sediment features and a positive effect on the local biodiversity and the associated species assemblages, the food web structure remained unaltered. Similarly, the engineering activity of eelgrass *Zostera noltii* did neither affect the planktonic nor the benthic food web structure in the Mondego estuary, Portugal (Baeta *et al.* 2009). A study by Botto *et al.* (2005) shows that the burrowing crab *Neohelice granulata*, an engineer in SW Atlantic coastal areas, modifies $\delta^{15}N$ values of sediments and primary producers by 3 to 7‰. Some consumers associated with the sedimentary environment reflected the enriched N values, but the overall food web structure in areas with and without the crab however remained largely unaltered.

Linking ecosystem engineering to food web structure and dynamics is still in its infancy and more empirical evidence, particularly for the marine environment, is needed. Additionally, based on the current study and existing literature, the effect of an ecosystem engineer on the structure of the food web is believed to depend on for example the engineering species, its position in the food web, and the type of the engineering activity. Nonetheless, the limited number of studies and the use of different indicators of trophic structure and dynamics, complicates the generalisation of the outcome of single case studies to ecosystem engineers in large.

Improving the conceptual models

The *L. conchilega* case study presented in this thesis shows that the currently existing conceptual models linking trophic and non-trophic interactions have some limitations. For instance, the relatively simple scenario's presented by Sanders *et al.* (2014) illustrate the importance of the trophic position of the engineer, and complex feedback interactions in influencing engineering effects on the food web stability (Fig. 3). Nevertheless, natural food webs are never as simple as a three-species food chain, and the *L. conchilega* food web shows that we cannot simply fit complex food webs into the

relatively easy conceptual models which we have at our disposal. Moreover, most conceptual models regarding ecosystem engineers are developed based on the terrestrial and fresh water food web theory (*e.g.* Kéfi *et al.* 2012). Marine food webs however are in general very distinct from their terrestrial and fresh water counterparts. The openness of marine ecosystems, lack of specialists, long life spans, and large size changes across the life histories of many marine species can collectively make marine food webs more highly connected than terrestrial and fresh water food webs (Link 2002). These differences imply that the food web theory needs to be modified to accommodate observations from marine systems (Link 2002). Hence, further modelling and empirical research is required to ascertain whether the conceptual links between ecosystem engineers and food webs hold for larger, more complex, and marine food webs. For example, Sanders *et al.* (2014) suggest that studying ecosystem engineering in food webs which are already well-characterised might be much easier than characterising food webs in systems where the engineer has been studied. Additionally, we might proceed to an integrated general understanding of how ecosystem engineering can alter food web structure and dynamics by building dynamic models that allow modelling and parameterisation of interactions between a limited number of food web species and engineers for real ecological systems (Sanders *et al.* 2014).

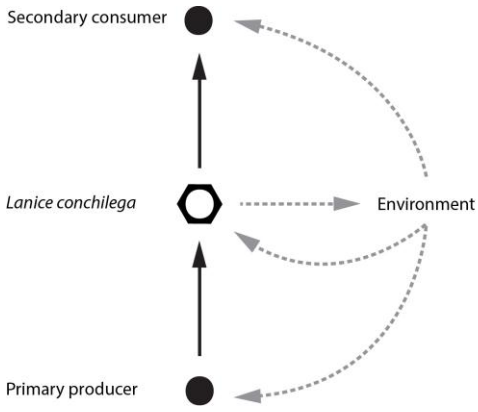


Figure 3. Simplified three-species food chain scenario exemplifying the importance of the trophic position of the engineer and complex feedbacks interactions in influencing engineer effects. The engineer Lanice conchilega*, which has an intermediate trophic position, can create feedbacks to the engineer density and the engineering activity via trophic effects (black arrows) and engineering effects (dotted arrows). Adapted from Sanders et al. (2014)*

Does the location matter?

In order to look beyond the local scale, assess the generality of our results and generate a solid base for the potential formulation of generic conservation measures in the future, the research conducted throughout this thesis was not confined to one single study site. The two selected study sites differed substantially in their environmental settings, which enabled us to make some generalisations about the structural and functional role of intertidal *L. conchilega* reefs regardless the variability in the surrounding environment (Chapter 1).

The positive feedback of *L. conchilega* on higher trophic levels of soft-bottom intertidal areas was shown to be applicable on locations with different environmental conditions (Chapter 2). The magnitude of the reef effect however seems to be influenced by the location of the reef. This finding is especially true for the macro- and epibenthic community and is mainly driven by the difference in the density of *L. conchilega* between the two sites. A higher tubeworm density will strengthen the structuring role of the reef since it provides a larger and more secure settlement surface of larval and post-larval macrobenthic organisms (Qian *et al.* 1999; Rabaut *et al.* 2007a), which on their turn promote the attraction of mobile epibenthic animals. Reef structures with higher tubeworm densities also facilitate the input of water column-derived carbon in the food web (Chapter 6), and therefore, locations with high density reefs will be able to sustain a higher macrofaunal abundance and biomass. Although the *L. conchilega* density is the most explaining factor, the location effect is amplified by location dependent environmental variables such as grain size and chlorophyll *a*. Unlike the macro- and epibenthos, the hyperbenthos is primarily structured by the availability of food at the different locations. The location did not influence the effect of *L. conchilega* on the overall structure and isotope niche of the food webs, which were shown to largely remain unaltered, except for an indirect engineering effect of the tubeworm on some consumer species (Chapter 5). In general, the structuring and functional role of a *L. conchilega* reef is assumed to be widespread, but can differ in strength depending on the density of the tubeworm reefs.

Conclusions

The general objective of this PhD thesis is to improve our understanding of the structural and functional role of reefs constructed by the ecosystem engineer *Lanice conchilega* in natural soft-substrate coastal ecosystems. This was achieved by studying the structural role of *L. conchilega* on the bentho-pelagic and the wader community associated with the reef habitats, investigating predator feedback on the ecosystem engineer, characterising the trophic interactions taking place within the reef habitats and comprehensively combining ecosystem engineering and trophic interactions, and quantifying carbon flows of a soft-bottom intertidal food web dominated by *L. conchilega* reefs.

The answers to the questions raised in the aims of the PhD thesis (Chapter 1), can be formulated as follows:

Objective 1: Investigating the effect of *L. conchilega* reefs on the density, diversity and composition of the bentho-pelagic and wader community of soft-bottom intertidal areas

The three investigated benthic assemblages (macro-, epi- and hyperbenthos) were positively affected by the presence of the *L. conchilega* reef structures. The magnitude of the effect depended on the link between the different benthic assemblages and the sediment and was thus largest for the macrobenthos and smallest for the hyperbenthos. The macro-, and epibenthos were mainly structured by the tubeworm density and macrobenthic food availability respectively, while the hyperbenthos was rather structured by biotic environmental variables. The high abundance, diversity and biomass of the associated macrofauna render the reef areas exceptionally important feeding grounds for waders in otherwise uniform habitats. As a result, wader densities in the reef exceeded densities in bare sand areas 47 times. These findings reinforce our knowledge on the central role that *L. conchilega* reefs play in linking benthic, pelagic and air-borne compartments in soft-bottom intertidal areas.

Objective 2: Investigating the effect of the predator *Crangon crangon* on the bioirrigation and feeding activity of an ecosystem engineer which is trophically coupled to the food web

The bioirrigation activity of a *L. conchilega* reef, equalling about 30 L m^{-2} d^{-1}, was shown to remain unaffected in the presence of the predatory shrimp *Crangon crangon*. The food uptake of the tubeworm was however about three times lower in the unrestricted presence of *C. crangon*, presumably due to the retraction of the worm's body and tentacles in its tube, induced by physical contact with the predator. Hence, *C. crangon* exerts a trophic pressure on the tubeworm which affects its feeding activity, but not its bioirrigation activity, which enables the tubeworm to maintain its functional role in soft-bottom intertidal areas, even under high predatory pressure.

Objective 3: Investigating ecosystem engineer-mediated effects on the structure and stability of a soft-bottom intertidal food web

Apart from the well-documented effects of *L. conchilega* on its environment and on the structure of the associated macrofaunal and wader community composition, the tubeworm reefs only had a minor influence on the food web structure of soft-bottom intertidal areas. The isotopic niche width of the consumer communities of reef and control areas were highly similar, implying that consumer taxa do not shift their diet when feeding in a *L. conchilega* reef. Besides, species packing and hence trophic redundancy were not affected, pointing to an unaltered stability of the food web in the presence of *L. conchilega*. As opposed to the strong impact of *L. conchilega* on the benthic assemblage, the ecosystem engineer's influence on the water column is probably too limited to substantially stir the global structure of the soft-bottom intertidal food web, which is mainly driven by water column-derived primary production.

Objective 4: Quantifying the carbon flows in a soft-bottom intertidal food web in the presence of ecosystem-engineered *L. conchilega* reefs

The carbon input into reef food webs was about 40 times higher compared to bare sand areas and was mainly derived from organic matter in the water column. The tubeworm

focussed the OM towards the reef produced in an area at least 15 times larger than the reef itself. Although most of the OM input towards these reefs was consumed by suspension feeding macrofauna, particularly *L. conchilega* itself, the worm was not an important source of carbon for other macrofaunal organisms. Rather, the structures created by *L. conchilega* acted as a trap of OM, sustaining the macrobenthic community and the fish and wading bird populations in the presence of the tubeworm, and resulting in much more diverse food webs.

Objective 5: Assessing whether the outcomes on the structural and functional role of *L. conchilega* on the ecosystem can be generalised beyond the local scale.

The structuring and functional role of a *L. conchilega* reef is assumed to be widespread, but can differ in strength depending on the location and the environmental conditions. The density of the tubeworm reef was identified as the most important variable in explaining the observed differences in the amount of carbon entering the food web and the macrofaunal abundance and biomass between locations.

General conclusion

This PhD thesis responds to the need for increasing our knowledge of the functional and structural role of ecosystem engineering in complex ecological communities. The ecosystem engineer *Lanice conchilega* facilitates the bentho-pelagic coupling in soft-bottom intertidal areas and was proven to strongly affect the faunal community composition, diversity, abundance and biomass, even under high predatory pressure. Notwithstanding this strong structural and functional role provided by the *L. conchilega* reefs, no direct effects on the overall structure or stability of the intertidal food web were detected. The magnitude of the links between different food web compartments however is modified by the increased carbon input in the presence of the reefs.

Future challenges

This thesis mainly deals with the autogenic engineering activities of *L. conchilega*. In the light of a holistic approach towards investigating the structural and functional role of the ecosystem engineer *L. conchilega*, the knowledge gained here should be

complemented with more insights in the allogenic engineering aspect and a focus on the interactions taking place below the sediment-water surface. The elevated bioirrigation in a *L. conchilega* reef as shown in this thesis gives rise to an increased organic matter mineralisation (Braeckman *et al.* 2010), which on its turn is assumed to be an important carbon source for smaller benthic animals (meiofauna) and microbiota. Although the interaction between ecosystem engineers and the diversity, abundance and activity (*i.e.* mineralisation, nitrification and denitrification) of microbiota has been intensively studied (*e.g.* Bertics and Ziebis 2009; Gilbertson *et al.* 2012), the link between *L. conchilega* and microbiota is until now largely unexplored. Braeckman *et al.* (2010) investigated the link between *L. conchilega* and microbial activity, however no studies on the ecosystem engineering activity of the tubeworm on the natural microbial community are available. Investigating the relationship between *L. conchilega* and the microbial community is recommended since it is assumed to be important in understanding the role of *L. conchilega* in determining food web structure, stability and dynamics. Moreover, Foshtomi *et al.* (2015) provided evidence that the relationship between microbial biodiversity and ecosystem functioning is modulated by macrofaunal activity in marine sediments. Therefore, it can be hypothesised that the bioirrigation activity of *L. conchilega* affects the microbial biodiversity-ecosystem functioning link.

Future research should focus on the effect of *L. conchilega* reefs on macrofauna, meiofauna and microbiota at the same time. In this study, meiofauna is for instance assumed to be the missing link in reconstructing the hump-shaped pattern characteristic for food webs that are structured such that top predators couple slow (detrital-based; including meiofauna) and fast (phytoplankton-based; mainly this study) energy channels.

Besides a need for the inclusion of smaller-sized benthos in qualitative and quantitative food web studies focussing on *L. conchilega* reefs, the avian compartment is lacking as well. This thesis provides evidence for the importance of *L. conchilega* reefs as feeding areas for wading birds, however the inclusion of avifauna in the current food web study was hampered by logistical constraints (*e.g.* the large size and the limited accessibility of

the sampling area of the BMSM). For future research, it can be recommended to investigate the interactions between avifauna and *L. conchilega* reefs by focussing on smaller sample areas (*e.g.* the intertidal area of Boulogne-sur-Mer) and make use of non-destructive sampling techniques such as the collection of bird faeces for genetic analysis and blood samples for stable isotope analysis.

In order to get a complete picture of the biotic and abiotic interactions taking place within the reef structures, both empirical and modelling efforts on different aspects of benthic ecosystem functioning are required. In line with the research done in this thesis, we recommend future studies to integrate diverse approaches and techniques ranging from large-scale field samplings and experimental field and laboratory setups, to the use of models to quantify biogeochemical cycling. Moreover, it would be interesting to adopt a similarly diverse approach for other ecosystem engineers and make a more justified and accurate comparison of the effect of ecosystem engineers on different aspects of the ecosystem.

ADDENDA

Addendum I

Appendices to Chapter 2

Appendix 1. Macrobenthic taxa list excluding strictly hyper-, or epibenthic organisms. Indication of the life history stage is given (ad = adult: juv = juvenile)

Taxon	Stage	Taxon	Stage
Actiniaria sp.	ad	*Macoma balthica*	ad
Ampharetinae sp.	ad	*Magelona johnstoni*	ad
Angulus tenuis	ad	*Magelona mirabilis*	ad
Aphelochaeta marioni	ad	*Malmgreniella arenicolae*	ad
Arenicola marina	ad	*Malmgreniella ljungmani*	ad
Autolytus sp.	ad	Malmgreniella sp.	ad
Bathyporeia elegans	ad	*Melita palmata*	ad
Bathyporeia pilosa	ad	*Monocorophium acherusicum*	ad
Bathyporeia sarsi	ad	Nematoda sp.	ad
Bivalvia sp.	juv	Nemertea sp.	ad
Capitella sp.	ad	*Nephtys caeca*	ad
Carcinus maenas	ad	*Nephtys cirrosa*	ad
Cerastoderma edule	ad	*Nephtys hombergii*	ad
Cirratulidae sp.	ad	*Nephtys kersivalensis*	ad
Corophium sp.	ad	Nereis sp.	ad
Cumacea sp.	ad	*Notomastus latericeus*	ad
Cumopsis goodsir	ad	Oligochaeta sp.	ad
Cumopsis longipes	ad	*Ophelia borealis*	ad
Donax vittatus	juv	Ophiuroidea sp.	juv
Ensis magnus	ad	*Pholoe minuta*	ad
Eocuma dollfusi	ad	*Phyllodoce mucosa*	ad
Eteone longa	ad	*Pirimela denticulata*	ad
Eumida sanguinea	ad	Polynoinae sp.	ad
Eurydice pulchra	ad	Portunidae sp.	ad
Gammarus crinicornis	ad	*Pygospio elegans*	ad
Glycera alba	ad	*Scolelepis squamata*	ad
Glycera lapidum	ad	*Scoloplos armiger*	ad
Glycera sp.	ad	*Sigalion mathildae*	ad
Glycera tridactyla	ad	Siphonoecetes sp.	ad
Harmothoe sp.	ad	*Sphaeroma monodi*	ad
Hediste diversicolor	ad	Spio sp.	ad
Hesionura elongata	ad	*Spiophanes bombyx*	ad
Heteromastus filiformis	ad	Syllidae sp.	ad
Idotea pelagica	ad	*Syllis gracilis*	ad
Idotea sp.	juv	*Tanaidacea sp.*	ad
Kurtiella bidentata	ad	*Urothoe poseidonis*	ad
Lanice conchilega	ad	Urothoe sp.	juv
Liocarcinus navigator	ad	*Venerupis philippinarum*	ad
Lumbrineris sp.	ad		

Appendix 2. Epibenthic taxa list excluding strictly macrobenthic taxa and taxa which were more efficiently caught with the hyperbenthic sledge (i.e. small sized crustaceans such as isopods and mysids; early postlaval fish; and jellyfish). Indication of the life history stage (based on Beyst et al. 2001b) is given (ad = adult: juv = juvenile)

Taxon	**Stage**	**Taxon**	**Stage**
Ammodytes tobianus	ad	*Palaemon serratus*	ad
Ammodytidae sp.	juv	*Pisidia longicornis*	ad
Aphia minuta	ad	*Platichthys flesus*	ad
Atherina presbyter	ad	*Pleuronectes platessa*	ad
Cancer pagurus	ad	Pleuronectidae sp.	ad
Carcinus maenas	ad	*Pomatoschistus lozanoi*	ad
Ciliata mustela	ad	*Pomatoschistus microps*	ad
Cottidae sp.	ad	*Pomatoschistus minutus*	ad
Crangon crangon	ad	*Pomatoschistus pictus*	ad
Dicentrarchus labrax	ad	*Porcellana platycheles*	ad
Diogenes pugilator	ad	Porifera sp.	ad
Echiichthys vipera	ad	*Portumnus latipes*	ad
Eualus occulus	ad	*Psammechinus miliaris*	ad
Eualus pusiolus	ad	*Scophthalmus rhombus*	ad
Liocarcinus marmoreus	ad	*Sepia officinalis*	ad
Liocarcinus sp.	ad	*Sepiola atlantica*	ad
Liocarcinus vernalis	ad	*Solea solea*	ad
Loligo sp.	ad	*Sprattus sprattus*	ad
Loligo vulgaris	ad	*Syngnathus rostellatus*	ad
Mytilus edulis	ad	Tunicata sp.	ad

Appendix 3. Hyperbenthic taxa list excluding strictly macrobenthic, larger epibenthic and sessile organisms. Indication of the life history stage (based on Beyst et al. 2001a) is given (ad = adult, juv = juvenile; la = larvae, me = megalopa, zo = zoea)

Taxon	Stage	Taxon	Stage	Taxon	Stage
Abludomelita gladiosa	ad	*Dexamine spinosa*	ad	*Monocorophium sextonae*	ad
Abludomelita obtusata	ad	*Diastylis bradyi*	ad	Mysidae sp.	ad
Achelia echinata	ad	*Diastylis lucifera*	ad	*Mysidopsis gibbosa*	ad
Achelia sp.	ad	Diastylis sp.	ad	*Neomysis integer*	ad
Allomelita pellucida	ad	*Endeis spinosa*	ad	*Nototropis falcatus*	ad
Ammodytes tobianus	juv	*Eocuma dollfusi*	ad	*Nototropis guttatus*	ad
Ammodytidae sp.	juv	*Ericthonius punctuatus*	ad	*Nototropis swammerdamei*	ad
Ammothella longipes	ad	Ericthonius sp.	ad	Nudibranchia sp.	ad
Amphipoda sp.	ad	*Eualus cranchii*	ad	*Nymphon brevirostre*	ad
Anilocra sp.	ad	Eualus sp.	ad	Ophiuroidea sp.	juv
Anoplodactylus pygmaeus	ad	*Euphausiidae sp.*	ad	*Palaemon elegans*	ad
Aoridae sp.	ad	*Eurydice pulchra*	ad	*Palaemon serratus*	ad
Apherusa ovalipes	ad	Galathea sp.	juv	*Pariambus typicus*	ad
Apherusa sp.	ad	Galatheidae sp.	me	*Philocheras fasciatus*	ad
Apseudopsis latreillii	ad	Gammarus sp.	ad	*Philocheras trispinosus*	ad
Astacilla longicornis	ad	*Gastrosaccus spinifer*	ad	*Phoxichilidium femoratum*	ad
Asterias rubens	juv	Gobiidae sp.	juv	*Phtisica marina*	ad
Athanas nitescens	ad	*Haustorius arenarius*	ad	*Pilumnus hirtellus*	ad
Atylus sp.	ad	*Heteromysis formosa*	ad	*Pinnotheres pisum*	ad
Atylus vedlomensis	ad	Hippolyte sp.	ad	Pisces sp.	juv
Bathyporeia elegans	ad	Hippolyte sp.	juv	*Pisidia longicornis*	ad
Bathyporeia pelagica	ad	*Hippolyte varians*	ad	*Pleurobrachia pileus*	ad
Bathyporeia pilosa	ad	Hippolytidae sp.	ad	Pleuronectiformes sp.	juv
Bathyporeia sarsi	ad	*Idotea balthica*	ad	*Pontocrates arenarius*	ad
Bathyporeia sp.	ad	*Idotea linearis*	ad	Porcellanidae sp.	juv
Bodotria scorpioides	ad	*Idotea metallica*	ad	*Portumnus latipes*	juv
Bougainvilliidae sp.	ad	*Idotea pelagica*	ad	Portunidae sp.	juv + zo
Calanoida sp.	ad	Idotea sp.	ad	*Praunus flexuosus*	ad
Calliopius laeviusculus	ad	*Iphimedia obesa*	ad	*Processa edulis crassipes*	ad
Callipallene brevirostris	ad	Jaera sp.	ad	Processa sp.	ad
Caprella linearis	ad	*Jassa herdmani*	ad	*Pseudoprotella phasma*	ad
Caridea sp.	juv	*Jassa marmorata*	ad	Pycnogonida sp.	ad
Cheirocratus intermedius	ad	Jassa sp.	ad	*Schistomysis kervillei*	ad
Cheirocratus sp.	ad	*Lekanesphaera monodi*	ad	*Schistomysis ornata*	ad
Clupeidae sp.	la	*Leptomysis lingvura*	ad	Schistomysis sp.	ad
Cnidaria sp.	ad	*Leptomysis mediterranea*	ad	*Schistomysis spiritus*	ad
Corophium arenarium	ad	*Leucothoe incisa*	ad	*Sepiola atlantica*	ad
Corophium sp.	ad	*Listriella picta*	ad	Siphonoecetes sp.	ad
Corophium volutator	ad	*Maerella tenuimana*	ad	*Siriella armata*	ad
Corynidae sp.	ad	Majidae sp.	juv	*Siriella clausii*	ad
Crangon crangon	juv	*Melita palmata*	ad	*Siriella jaltensis*	ad
Crangonidae sp.	juv	*Mesopodopsis slabberi*	ad	*Stenothoe marina*	ad
Ctenophora sp.	ad	Microprotus sp.	ad	*Sunamphitoe pelagica*	ad
Cyclopoida sp.	ad	*Monocorophium acherusicum*	ad	*Urothoe poseidonis*	ad
Decapoda sp.	me	*Monocorophium insidiosum*	ad		

Appendix 4. Three-factor univariate Permanova main and pair-wise tests results for the environmental variables. Reef (reef vs. control), Location (BMSM vs. Boulogne) and Period (spring vs. autumn) were fixed factors. Analyses were based on an Euclidian distance resemblance matrix and performed on untransformed data (except for the total organic matter, TOM, which was fourth-root transformed). Only significant results including the factor Reef are shown. In case of significant differences (p < 0.05) p values are in bold

	Reef x Location x Period			Reef x Location			Reef x Period		
Main test	MS	pseudo-F	*p* value	MS	pseudo-F	*p* value	MS	pseudo-F	*p* value
Median grain size (*µ*m)	1582.3	5.27	**0.026**	—	—	—	—	—	—
Mud content (%)	—	—	—	141.8	11.8	**0.0001**	—	—	—
MPB (µg.g^{-1} sediment)	—	—	—	0.19	5.67	**0.016**	34.56	8.74	**0.0025**
SPM (mg.L^{-1} water)	—	—	—	64254	16.86	**0.0012**	64254	16.86	**0.0012**
Chl*a* (µg.L^{-1} water)	—	—	—	—	—	—	272.12	4.88	**0.0375**

	Reef			Location			Period		
Main test	MS	pseudo-F	*p* value	MS	pseudo-F	*p* value	MS	pseudo-F	*p* value
Total organic matter (%)	0.18	17.41	**0.0001**	0.58	55.94	**0.0001**	0.07	6.44	**0.008**

	Reef x Location x Period				Reef x Location		Reef x Period	
Pair-wise test	BMSM - S	BMSM - A	Boul - S	Boul - A	BMSM	Boul	Spring	Autumn
Median grain size (*µ*m)	**0.0001**	**0.0002**	0.1991	0.9474	—	—	—	—
Mud content (%)	—	—	—	—	**0.0001**	0.1275	—	—
MPB (µg.g^{-1} sediment)	—	—	—	—	0.5298	**0.0014**	**0.0007**	0.9467
SPM (mg.L^{-1} water)	—	—	—	—	**0.0163**	**0.03**	—	—
Chl*a* (µg.L^{-1} water)	—	—	—	—	—	—	0.0646	**0.0096**

Appendix 5. Three-factor univariate Permanova main and pair-wise tests results for the macro-, epi-, and hyperbenthic community descriptors. Reef (reef vs. control), Location (BMSM vs. Boulogne) and Period (spring vs. autumn) were fixed factors. Analyses were based on an Euclidian distance resemblance matrix and performed on untransformed data (except for the macrobenthic species abundance N and the epibenthic species richness S which were fourth-root transformed). Only significant results including the factor Reef are shown. In case of significant differences (p < 0.05) p values are in bold

	Main test Reef x Location			**Pair-wise test** BMSM	Boulogne
Macrobenthos	MS	pseudo-F	*p* value	*p* value	*p* value
Species abundance *N*	88.97	90.58	**0.0001**	**0.0001**	**0.0001**
Species richness *S*	361.25	47.19	**0.0001**	**0.0001**	**0.0001**
Shannon diversity index *H'*	5.32	27.61	**0.0001**	0.1249	**0.0001**
Pielou's eveness index *J'*	0.05	5.65	**0.021**	**0.0001**	**0.0001**

	Main test Reef x Period			**Pair-wise test** Spring	Autumn	**Main test** Reef x Location x Period			**Pair-wise test** BMSM - S	BMSM - A	Boul - S	Boul - A
Epibenthos	MS	pseudo-F	*p* value	*p* value	*p* value	MS	pseudo-F	*p* value	*p* value	*p* value	*p* value	*p* value
Species abundance *N*	—	—	—	—	—	$1.14x10^6$	4.87	**0.0259**	**0.032**	0.7008	0.1993	**0.0283**
Species richness *S*	0.1	5.93	**0.0243**	**0.0049**	0.7291	—	—	—	—	—	—	—
Shannon diversity *H'*	—	—	—	—	—	0.65	6.81	**0.0142**	0.5205	0.7038	0.2989	**0.0284**
Pielou's eveness index *J'*	—	—	—	—	—	0.19	9.69	**0.0056**	0.1161	0.2046	0.8062	**0.0273**

	Main test Reef			**Main test** Location			**Main test** Period		
Hyperbenthos	MS	pseudo-F	*p* value	MS	pseudo-F	*p* value	MS	pseudo-F	*p* value
Species abundance *N*	$7.83x10^6$	6.48	**0.0107**	—	—	—	—	—	—
Species richness *S*	—	—	—	1210.3	30.11	**0.0001**	463	11.52	**0.0048**
Shannon diversity index *H'*	—	—	—	—	—	—	—	—	—
Pielou's eveness index *J'*	0.14	4.93	**0.0421**	—	—	—	—	—	—

*Appendix 6. Sequential tests of the distance-based linear model (DistLM) analyses on the macro-, epi-, and hyperbenthic communities. Analyses were run on a Bray-Curtis resemblance matrix of fourth-root transformed data. Both environmental (grain size, mud content, TOM, MPB, Chl a and SPM) and biotic (*L. conchilega *density, macrobenthic N, S and H') variables were included and (log or square-root) transformed if needed*

Variable	Adj. R^2	Pseudo-F	*p value*	Prop.	Cumul
Macrobenthos					
L. conchilega dens.	0.14004	13.865	0.0001	0.15093	0.15093
Mud content	0.22105	9.1122	0.0001	0.08984	0.24077
Grain size	0.25631	4.6505	0.0003	0.04377	0.28455
SPM	0.28844	4.4322	0.0002	0.03992	0.32447
TOM	0.30225	2.4837	0.0112	0.02193	0.34641
Chl *a*	0.31378	2.2431	0.022	0.01948	0.36589
MPB	0.31632	1.2716	0.2434	0.011	0.3769
Epibenthos					
SPM	0.13645	5.1084	0.0002	0.16967	0.16967
Macro H	0.22836	3.9778	0.0029	0.11805	0.28772
Macro *N*	0.43200	9.6046	0.0001	0.20982	0.49754
Chl *a*	0.52240	5.3532	0.0001	0.09833	0.59588
TOM	0.55197	2.4521	0.0124	0.04225	0.63813
Grain size	0.58565	2.7069	0.0072	0.04313	0.68127
Hyperbenthos					
TOM	0.12465	4.4176	0.0002	0.16112	0.16112
Chl *a*	0.25446	5.0045	0.0002	0.15546	0.31658
SPM	0.43174	7.8636	0.0001	0.18619	0.50277
Grain size	0.50219	3.9719	0.0001	0.08238	0.58516
MPB	0.54569	2.915	0.0002	0.05517	0.64034
Macro *S*	0.57696	2.4043	0.0097	0.04237	0.68272
Macro *N*	0.59030	1.5861	0.1046	0.02707	0.70979

Addendum II

Appendices to Chapter 5

Appendix 1. Mean stable isotope values of the consumer clusters of the Bay of the Mont Saint-Michel (BMSM). Classification of consumer taxa with similar food uptake ($\delta^{13}C$) and trophic level ($\delta^{15}N$) for different combinations of sampling area and period in the BMSM, based on agglomerative hierarchical cluster analyses and similarity profile (SIMPROF) permutation tests. Cluster names match the clusters defined in the $\delta^{13}C$ - $\delta^{15}N$ biplot of the BMSM (Figure 2). For each of the clusters, the mean $\delta^{13}C$ and $\delta^{15}N$ values (±SD) are displayed, as well as the taxonomic composition and the number of replicates per taxon (n)

Spring Reef					Control				
Cluster	$\delta^{13}C \pm SD$	$\delta^{15}N \pm SD$	Species	n	Cluster	$\delta^{13}C \pm SD$	$\delta^{15}N \pm SD$	Species	n
1A	-15.23 ± 0.17	15.64 ± 0.21	Pomatoschistus sp.	4	1B	-14.47 ± 1.04	13.93 ± 1.06	*Buccinum undatum*	1
2A	-14.38 ± 0.39	14.02 ± 0.11	*Crangon crangon*	4				*Crangon crangon*	4
3A	-17.74 ± 0.50	12.88 ± 0.59	*Schistomysis kervillei*	4				*Dicentrarchus labrax*	1
			Lanice conchilega	1				Pomatoschistus sp.	4
			Polynoinae sp.	3				*Nepthys cirrosa*	1
4A	-15.71 ± 0.24	9.64 ± 1.22	*Macoma balthica*	3	2B	-18.57 ± 1.60	9.81 ± 1.90	Corophium sp.	1
			Nephtys sp.	1				Gammarus sp.	4
5A	18.75 ± 0.59	11.03 ± 0.68	*Schistomysis kervillei*	1				*Gastrosaccus spinifer*	3
			Schistomysis spiritus	2				Idotea linearis	1
			Lanice conchilega	3				*Schistomysis kervillei*	4
6A	-19.33 ± 1.16	6.80 ± 1.94	*Athanas nitescens*	1				*Syngnathus rostellatus*	3
			Gammarus sp.	5				*Cerastoderma edule*	4
			Lekanesphaera levii	2				*Macoma balthica*	3
			Abludomelita obtusata	1				Oligochaeta sp.	1
			Melita sp.	1					
			Schistomysis spiritus	1					
			Bathyporeia elegans	1					
			Cerastoderma edule	1					
7A	-23.45	2.62	*Lekanesphaera levii*	1					

Autumn Reef					Control				
Cluster	$\delta^{13}C \pm SD$	$\delta^{15}N \pm SD$	Species	n	Cluster	$\delta^{13}C \pm SD$	$\delta^{15}N \pm SD$	Species	n
1C	-15.53 ± 0.25	14.71 ± 0.34	*Atherina presbyter*	1	1D	-17.92	15.62	*Loligo vulgaris*	1
			Loligo vulgaris	3	2D	-15.54 ± 0.17	14.81 ± 0.16	Pomatoschistus sp.	4
			Pomatoschistus sp.	4	3D	-14.80 ± 0.34	13.33 ± 0.20	*Crangon crangon*	1
2C	-16.95 ± 0.04	13.89 ± 0.75	*Palaemon serratus*	1				*Pleuronectes platessa*	2
			Loligo vulgaris	1				*Scoloplos armiger*	1
3C	-14.85 ± 0.39	13.99 ± 0.49	*Crangon crangon*	1	4D	-13.56 ± 0.47	13.15 ± 0.29	*Crangon crangon*	2
			Dicentrarchus labrax	1				Liocarcinus sp.	2
			Solea solea	7				*Platichthys flesus*	1
4C	-12.68 ± 0.62	13.27 ± 0.40	*Crangon crangon*	3				*Portumnus latipes*	2
5C	-16.48 ± 1.25	10.48 ± 1.14	*Buccinum undatum*	3	5D	-11.75	12.12	*Crangon crangon*	1
			Carcinus maenas	1	6D	-17.65 ± 1.54	10.72 ± 1.70	*Diogenes pugilator*	4
			Diogenes pugilator	3				*Pleuronectes platessa*	1
			Rhizostoma pulmo	1				Pomatoschistus sp.	1
			Gammarus sp.	2				*Corophium volutator*	1
			Idotea balthica	2				Diastylis sp.	1
			Idotea linearis	3				Gammarus sp.	4
			Arenicola marina	1				*Gastrosaccus spinifer*	2
			Cerastoderma edule	4				*Idotea linearis*	1
			Lanice conchilega	4				*Lekanesphaera levii*	2
			Macoma balthica	4				*Mesopodop sis slabberi*	3
			Nephtys cirrosa	3				*Philocheras trispinosus*	2
			Nereis sp.	1				Processa sp.	2
6C	-19.76 ± 0.74	8.52 ± 0.74	Gammarus sp.	2				*Schistomysis spiritus*	3
								Syngnathus rostellatus	1
								Eualus cranchii	4
								Macoma balthica	2
								Nephtys cirrosa	3
								Scoloplos armiger	1

Appendix 2. Mean stable isotope values of the consumer clusters of the intertidal area of Boulogne-sur-Mer. Classification of consumer taxa with similar food uptake ($\delta^{13}C$) and trophic level ($\delta^{15}N$) for different combinations of sampling area and period in Boulogne-sur-Mer, based on agglomerative hierarchical cluster analyses and similarity profile (SIMPROF) permutation tests. Cluster names match the clusters defined in the $\delta^{13}C$ - $\delta^{15}N$ biplot of Boulogne (Figure 3). For each of the clusters, the mean $\delta^{13}C$ and $\delta^{15}N$ values (±SD) are displayed, as well as the taxonomic composition and the number of replicates per taxon (n)

Spring Reef					Control				
Cluster	$\delta^{13}C \pm SD$	$\delta^{15}N \pm SD$	Species	n	Cluster	$\delta^{13}C \pm SD$	$\delta^{15}N \pm SD$	Species	n
1E	-18.18 ± 2.10	12.19 ± 2.16	*Carcinus maenas*	3	1F	-17.44 ± 1.30	11.90 ± 2.81	Ammodytidae sp.	4
			Carcinus maenas juv.	1				*Carcinus maenas*	4
			Crangon crangon	4				*Psammechinus miliaris*	1
			Pleuronectes platessa	1				Crangon crangon juv.	3
			Pomatoschistus sp.	2				*Gastrosaccus spinifer*	1
			Gammarus sp.	1				*Mesopodopsis slabberi*	2
			Mesopodopsis slabberi	4				Pleuronectidae sp.	1
			Pleuronectidae juv.	4				*Schistomysis kervillei*	1
			Pleuronectidae larvae	4				*Syngnathus rostellatus*	1
			Syngnathus rostellatus	1				*Nepthys cirrosa*	2
			Lanice conchilega	3					
			Pholoe minuta	1					
			Phyllodoce mucosa	1					
			Polynoinae sp.	4					
Autumn Reef					Control				
Cluster	$\delta^{13}C \pm SD$	$\delta^{15}N \pm SD$	Species	n	Cluster	$\delta^{13}C \pm SD$	$\delta^{15}N \pm SD$	Species	n
1G	-16.71 ± 1.01	13.99 ± 0.78	*Carcinus maenas*	4	1H	-16.59 ± 1.10	13.72 ± 0.90	Actiniaria sp.	1
			Crangon crangon	4				Ammodytidae sp.	1
			Dicentrarchus labrax	4				*Buccinum undatum*	1
			Echiichthys vipera	1				*Carcinus maenas*	5
			Pleuronectes platessa	2				*Crangon crangon*	4
			Pomatoschistus microps	4				*Dicentrarchus labrax*	2
			Pomatoschistus sp.	4				*Echiichthys vipera*	1
			Scopthalmus rhombus	1				Liocarcinus sp.	3
			Sprattus sprattus	1				*Pleuronectes platessa*	7
			Arenicola marina	1				*Pomatoschistus microps*	3
			Glycera alba	2				Pomatoschistus sp.	10
			Lumbrineris sp.	1				*Scopthalmus rhombus*	4
			Phyllodoce mucosa	1				*Gastrosaccus spinifer*	1
2G	-17.70 ± 0.13	11.02 ± 0.89	*Lanice conchilega*	1				*Praunus flexuosus*	1
			Nephtys cirrosa	2				*Eualus cranchii*	1
			Phyllodoce mucosa	1				*Nephtys cirrosa*	3
			Urothoe poseidonis	1	2H	-21.30 ± 0.92	9.16 ± 1.72	Liocarcinus sp. juv.	1
3G	-20.30 ± 0.44	9.98 ± 0.09	*Lanice conchilega*	1				Gammarus sp.	1
			Urothoe poseidonis	2				*Gastrosaccus spinifer*	2
4G	-19.23 ± 0.15	10.33 ± 0.11	*Lanice conchilega*	2				*Nototropis swammerdamei*	3
			Notomastus sp.	1				*Eurydice pulchra*	1
			Urothoe sp. juv.	4					
5G	-19.72	10.71	*Urothoe poseidonis*	1					
6G	-23.29	8.2	Venerupis sp.	1					

Addendum III

Appendices to Chapter 6

Appendix 1. Site-specific biomass data of the different compartments (primary producers and macrofaunal consumer species) for the eight food webs. The biomass stocks are expressed as mmol C m^{-2}

		BMSM				Boulogne			
		Autumn		Spring		Autumn		Spring	
Compartment	Abbr.	Control	Reef	Control	Reef	Control	Reef	Control	Reef
Microphytobenthos (Primary producer)	MICPHY	43.70	38.40	18.21	41.52	11.32	21.98	32.23	144
Phytoplankton (Primary producer)	PHYTOP	2.17	1.19	15.14	16.36	1.10	1.24	7.19	14.37
Lanice conchilega (Polychaeta)	LANCON	0	6189	0	3203	0	18225	0	22212
Arenicola marina (Polychaeta)	AREMAR	17.81	1752	0	284	0	378	4.56	0
Capitella capitata (Polychaeta)	CAPCAP	0.0733	0.5892	0.2886	0.8240	0	15.55	0	8.41
Eumida sanguinea (Polychaeta)	EUMSAN	0	1	0	3.29	0	6.77	0	112
Heteromastus filiformis (Polychaeta)	HETFIL	0.9427	0.8031	0.2619	1.12	0	15.28	0	14.52
Nephtys cirrosa (Polychaeta)	NEPCIR	6.92	0.9427	13.50	0.7856	11.57	9.95	14.11	20.16
Nephtys hombergii (Polychaeta)	NEPHOM	3.30	65.96	0	40.33	0	0	0.2619	0
Nereis sp. (Polychaeta)	NERESP	0	0	0	0	0	18.70	0	22.78
Notomastus latericeus (Polychaeta)	NOTLAT	0	1	0	0.0524	0	1.45	0	8.48
Phyllodoce mucosa (Polychaeta)	PHYMUC	0	0.3142	0	1.31	0	4.90	0	28.01
Pygospio elegans (Polychaeta)	PYGELE	0.1047	0.1571	0	0.0471	0	15.01	0	0.8607
Scolelepis squamata (Polychaeta)	SCOSQU	0	0.1047	0	0	0	0	0.8249	0
Scoloplos armiger (Polychaeta)	SCOARM	13.09	0	5.50	0	0	0	0	0
Spio sp. (Polychaeta)	SPIOSP	0.0419	0.0209	0.2933	0.0524	0.0524	0.0209	0.2374	0.6285
Spiophanes bombyx (Polychaeta)	SPIBOM	0.1571	0	0	0	0.5106	1.61	0	1.41
Tellina tenuis (Bivalvia)	TELTEN	22.58	0	12.76	0	0	23.61	0	0
Cerastoderma edule (Bivalvia)	CEREDU	0	7535	165	4380	0	0	0	0
Macoma balthica (Bivalvia)	MACBAL	44.16	1477	47.82	610	0	0.6104	0	0
Eurydice pulchra (Isopoda)	EURPUL	0	0	0	0	0.7110	0.0970	0.0970	4.75
Idotea balthica (Isopoda)	IDOBAL	0.1409	0.0470	0	0	0	0	0	0
Idotea linearis (Isopoda)	IDOLIN	0.0046	0.0563	0.0498	0.0528	0	0	0	0
Lekanesphaera monodi (Isopoda)	LEKMON	0	2.04	0.5845	0.7758	0	0.3876	0	0
Gammarus sp. (Amphipoda)	GAMMSP	0.0209	0.0189	0.1334	0.0879	0.0006	0.0087	0	0.0031
Urothoe poseidonis (Amphipoda)	UROPOS	0.8310	3.79	0.7031	3.27	0.1278	28.69	0	35.85
Urothoe sp. juvenile (Amphipoda)	UROJUV	0	0.2216	0.4261	0.5199	0	19.29	0.0639	3.79
Gastrosaccus spinifer (Mysida)	GASSPI	0.0011	0.0064	0.0547	0.0008	0.0014	0.0001	0.0031	0.0005
Mesopodopsis slabberi (Mysida)	MESSLA	0.0800	0.7721	0	0.0040	0	0.0006	0.0108	0.4817
Schistomysis kervillei (Mysida)	SCHKER	0.0205	0.0053	0.1856	0.0885	0.0002	0	0.0006	0.0120
Schistomysis spiritus (Mysida)	SCHSPI	0.0826	0.0474	0.0024	0.0042	0	0.0003	0.0004	0.0002
Carcinus maenas (Decapoda)	CARMAE	0.0101	25.21	0.0079	0.6164	1.15	7846	187	848
Crangon crangon (Decapoda)	CRACRA	0.9724	1.41	0.5870	2.13	1.75	48.66	0.3323	4.09
Diogenes pugilator (Decapoda)	DIOPUG	0.0073	0.0028	0	0	0	0	0	0
Palaemon serratus (Decapoda)	PALSER	0	0.0084	0	0	0	0.0201	0	0
Pisidia longicornis (Decapoda)	PISLON	0.0005	0.0016	0	0.0001	0.0004	0	0	0
Portumnus latipes (Decapoda)	PORLAT	0.0734	0	0.0203	0.0008	0	0	0	0
Loligo vulgaris (Cephalopoda)	LOLVUL	0.1153	0.0635	0	0	0	0	0	0
Dicentrarchus labrax (Osteichthyes)	DICLAB	0	0	0.1372	0.5009	0.1173	0.2075	0	0.1013
Echiichthys vipera (Osteichthyes)	ECHVIP	0.0054	0	0	0	0	0.4902	0	0
Platichthys flesus (Osteichthyes)	PLAFLE	0	0	0	0.2039	0.2589	0.4539	0	0
Pleuronectes platessa (Osteichthyes)	PLEPLA	0.1547	0	0.0721	0.6088	0.5657	2.56	0.2155	0
Pomatoschistus lozanoi (Osteichthyes)	POMLOZ	0.5128	0.3179	0.1003	1.39	0.0635	0.0363	0	0
Pomatoschistus microps (Osteichthyes)	POMMIC	0.0386	0.9581	0.0510	0.0627	0.0416	1.67	0	0.1949
Pomatoschistus minutus (Osteichthyes)	POMMIN	0.1072	0.1708	0	0.0852	2.46	6.62	0	0.0240
Scophthalmus rhombus (Osteichthyes)	SCORHO	0	0	0	0	0.1236	0.3678	0.1246	0
Solea solea (Osteichthyes)	SOLSOL	2.10	1.79	0	0	0	0	0	0
Syngnathus rostellatus (Osteichthyes)	SYNROS	0	0	0.0028	0.0035	0	0.0104	0	0.0009
Total biomass		160	17102	282	8593	32	26695	247	23485

Appendix 2. Site-specific stable isotope data ($\delta^{13}C$, ‰) of the different compartments (primary producers and macrofaunal consumer species) for the eight food webs

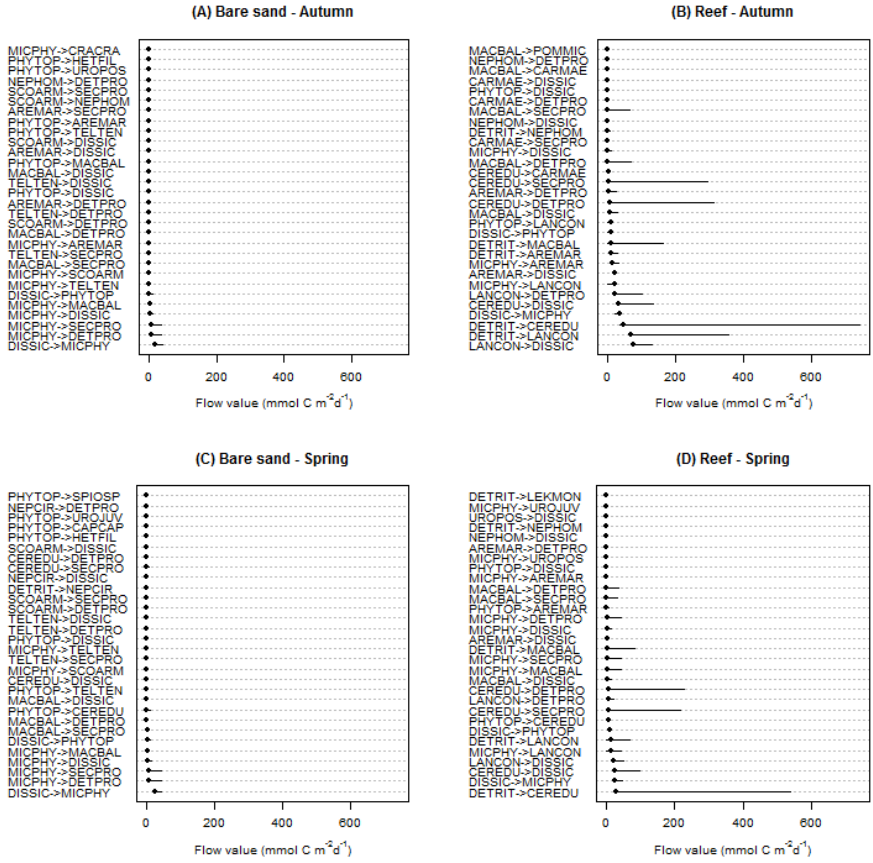
		BMSM				Boulogne			
		Autumn		Spring		Autumn		Spring	
Compartment	Abbr.	Control	Reef	Control	Reef	Control	Reef	Control	Reef
Microphytobenthos (Primary producer)	MICPHY	-14.08	-11.17	-9.74	-6.84	-19.58	-15.38	-12.67	-16.51
Phytoplankton (Primary producer)	PHYTOP	-21.29	-21.39	-21.40	-21.48	-23.53	-23.63	-21.15	-20.51
Lanice conchilega (Polychaeta)	LANCON	—	-17.40	—	-18.55	—	-18.99	—	-18.02
Arenicola marina (Polychaeta)	AREMAR	-15.94*	-15.94	—	-15.94*	—	-17.27	-17.27*	—
Capitella capitata (Polychaeta)	CAPCAP	-16.93†	-16.93†	-16.93†	-16.93†	—	-16.93†	—	-16.93†
Eumida sanguinea (Polychaeta)	EUMSAN	—	-16.27†	—	-16.27†	—	-16.27†	—	-16.27†
Heteromastus filiformis (Polychaeta)	HETFIL	-19.27‡	-19.27‡	-19.27‡	-19.27‡	—	-19.27‡	—	-19.27‡
Nephtys cirrosa (Polychaeta)	NEPCIR	-16.56	-16.21	-15.88	-15.88*	-16.33	-17.61	-16.59	-16.59*
Nephtys hombergii (Polychaeta)	NEPHOM	-15.40*	-15.40*	—	-15.40	—	—	-15.40*	—
Nereis sp. (Polychaeta)	NERESP	—	—	—	—	—	-15.41†	—	-15.41†
Notomastus latericeus (Polychaeta)	NOTLAT	—	-19.27*	—	-19.27*	—	-19.27	—	-19.27*
Phyllodoce mucosa (Polychaeta)	PHYMUC	—	-18.10*	—	-18.23*	—	-18.10	—	-18.23
Pygospio elegans (Polychaeta)	PYGELE	-16.25‡	-16.25‡	—	-16.25‡	—	-16.25‡	—	-16.25‡
Scolelepis squamata (Polychaeta)	SCOSQU	—	-16.25‡	—	—	—	—	-16.25‡	—
Scoloplos armiger (Polychaeta)	SCOARM	-16.25	—	-16.25*	—	—	—	—	—
Spio sp. (Polychaeta)	SPIOSP	-16.25‡	-16.25‡	-16.25‡	-16.25‡	-16.25‡	-16.25‡	-16.25‡	-16.25‡
Spiophanes bombyx (Polychaeta)	SPIBOM	-16.25	—	—	—	-16.25	-16.25	—	-16.25
Tellina tenuis (Bivalvia)	TELTEN	-15.32‡	—	-15.94‡	—	—	-15.32‡	—	—
Cerastoderma edule (Bivalvia)	CEREDU	—	-17.03	-18.50	-18.42	—	—	—	—
Macoma balthica (Bivalvia)	MACBAL	-15.32	-15.32	-15.94	-15.81	—	-15.32*	—	—
Eurydice pulchra (Isopoda)	EURPUL	—	—	—	—	-20.70	-20.70*	-20.70*	-20.70*
Idotea balthica (Isopoda)	IDOBAL	-17.52*	-17.52	—	—	—	—	—	—
Idotea linearis (Isopoda)	IDOLIN	-16.61	-15.49	-19.39	-19.39*	—	—	—	—
Lekanesphaera monodi (Isopoda)	LEKMON	—	-17.63*	-21.22*	-21.22	—	-17.63*	—	—
Gammarus sp. (Amphipoda)	GAMMSP	-19.17	-18.99	-20.49	-19.71	-21.07	-21.07*	—	-19.48
Urothoe poseidonis (Amphipoda)	UROPOS	-19.66*	-19.66*	-19.66*	-19.66*	-19.66*	-19.66	—	-19.66*
Urothoe sp. juvenile (Amphipoda)	UROJUV	—	-19.21*	-19.21*	-19.21*	—	-19.21	-19.21*	-19.21*
Gastrosaccus spinifer (Mysida)	GASSPI	-18.57	-18.57*	-19.76	-19.76*	-20.78	-20.78*	-17.62	-17.62*
Mesopodopsis slabberi (Mysida)	MESSLA	-17.54	-17.54*	—	-17.54*	—	-16.86*	-17.23	-16.86
Schistomysis kervillei (Mysida)	SCHKER	-17.59*	-18.05*	-17.59	-18.05	-16.91*	—	-16.91	-16.91*
Schistomysis spiritus (Mysida)	SCHSPI	-16.61	-16.61*	-18.78*	-18.78	—	-16.61*	-18.78*	-18.78*
Carcinus maenas (Decapoda)	CARMAE	-18.17*	-18.17	-18.17*	-18.17*	-16.62	-16.49	-16.08	-16.05
Crangon crangon (Decapoda)	CRACRA	-13.14	-13.02	-13.30	-14.38	-16.39	-15.99	-17.13*	-17.13
Diogenes pugilator (Decapoda)	DIOPUG	-16.74	-15.59	—	—	—	—	—	—
Palaemon serratus (Decapoda)	PALSER	—	-16.91	—	—	—	-16.91*	—	—
Pisidia longicornis (Decapoda)	PISLON	-16.74‡	-15.59‡	—	-15.59‡	-16.74‡	—	—	—
Portumnus latipes (Decapoda)	PORLAT	-13.70	—	-13.70*	-13.70*	—	—	—	—
Loligo vulgaris (Cephalopoda)	LOLVUL	-17.92	-16.05	—	—	—	—	—	—
Dicentrarchus labrax (Osteichthyes)	DICLAB	—	—	-14.12	-14.12	-16.54	-16.73	—	-16.73*
Echiichthys vipera (Osteichthyes)	ECHVIP	-17.31*	—	—	—	—	-16.58	—	—
Platichthys flesus (Osteichthyes)	PLAFLE	—	—	—	-13.99*	-13.99*	-13.99*	—	—
Pleuronectes platessa (Osteichthyes)	PLEPLA	-15.51	—	-15.51*	-15.51*	-15.96	-16.29	-24.99*	—
Pomatoschistus lozanoi (Osteichthyes)	POMLOZ	-15.77	-15.32	-15.29	-15.23	-16.76	-16.38	—	—
Pomatoschistus microps (Osteichthyes)	POMMIC	-15.77	-15.32	-15.29	-15.23	-16.76	-16.38	—	-17.96
Pomatoschistus minutus (Osteichthyes)	POMMIN	-15.77	-15.32	—	-15.23	-16.76	-16.38	—	-17.96
Scophthalmus rhombus (Osteichthyes)	SCORHO	—	—	—	—	-16.58	-17.24	-16.58*	—
Solea solea (Osteichthyes)	SOLSOL	-14.67*	-14.67	—	—	—	—	—	—
Syngnathus rostellatus (Osteichthyes)	SYNROS	—	—	-18.27	-18.27*	—	-17.59*	—	-17.59

* $\delta^{13}C$ value adopted from a different sampling area, season or location

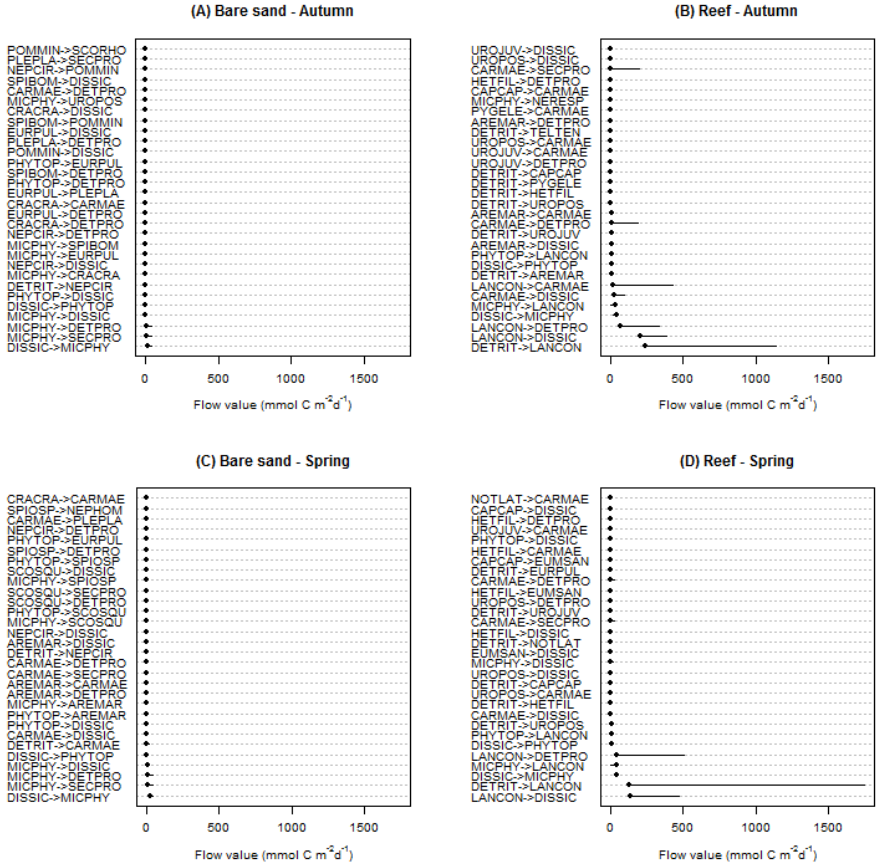
‡ $\delta^{13}C$ value adopted from a taxonomically related species

† $\delta^{13}C$ value adopted from the reference situation of a ^{13}C pulse-chase experiment (De Smet *et al.* under review)

Appendix 3. Plot with the parsimonious (simplest) solutions encompassed by their range for the 30 highest flows in the different food webs of the intertidal area of the Bay of the Mont Saint-Michel (BMSM) returned by the food web model



Appendix 4. Plot with the parsimonious (simplest) solutions encompassed by their range for the 30 highest flows in the different food webs of the intertidal area of Boulogne-sur-Mer returned by the food web model



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PUBLICATION LIST

Publication list

A1 – Peer reviewed articles

1. De Smet B., Godet L., Fournier J., Desroy N., Jaffré M., Vincx M., Rabaut M. (2013) Feeding grounds for waders in the Bay of the Mont Saint-Michel (France): the *Lanice conchilega* reef serves as an oasis in the tidal flats. *Marine biology*, **160**(4), 751-761.

2. De Smet B., D'Hondt A.-S., Verhelst P., Fournier J., Godet L., Desroy N., Rabaut M., Vincx M., Vanaverbeke J. (2015) Biogenic reefs affect multiple components of intertidal soft-bottom benthic assemblages: the *Lanice conchilega* case study. *Estuarine, Coastal and Shelf Science*, 152(0), 44-55.

3. De Smet B., Fournier J., De Troch M., Vincx M., Vanaverbeke J. (in press) Integrating ecosystem engineering and food web ecology: testing the effect of biogenic reefs on the food web of an soft-bottom intertidal area. *PLoS ONE*,

4. De Smet B., Braeckman U., Soetaert K., Vincx M., Vanaverbeke J. (under review) Predator effects on the feeding and bioirrigation activity of ecosystem-engineered *Lanice conchilega* reefs. *Journal of experimental marine biology and ecology*,

Articles in preparation

1. De Smet B., van Oevelen D., Vincx M., Vanaverbeke J., Soetaert K. (in prep.) *Lanice conchilega* structures carbon flows in soft-bottom intertidal areas.

2. Alves R.M.S., van Colen C., Vincx M., Vanaverbeke J., De Smet B., Guarini J.-M., Bouma T. (in prep.) Growing reefs: exploring the relationship between population and sediment dynamics on the formation of sandmason aggregations.

Poster presentations

1. De Smet, B., Vanaverbeke, J., Vincx, M. (2013). 'The importance of *Lanice conchilega* reefs in trophic linkages on intertidal sandy beaches', *in*: Mees, J. *et al.* (Ed.) (2013). Book of abstracts – VLIZ Young Marine Scientists' Day. Brugge, Belgium, 15 February 2013. VLIZ Special Publication, 63: pp. 36.

2. De Smet, B., D'Hondt, A.-S., Verhelst, P., Fournier, J., Godet, L., Desroy, N., Rabaut, M., Vincx, M., Vanaverbeke, J. (2014). 'New insights in the structuring role of *Lanice conchilega* reefs in intertidal food webs: a focus on epi- and hyperbenthos', *in*: Mees, J. *et al.* (Ed.) (2014). Book of abstracts – VLIZ Young Scientists' Day. Brugge, Belgium, 7 March 2014. VLIZ Special Publication, 67: pp. 40.

3. De Smet, B., Braeckman, U., Rabaut, M., Vincx, M., Vanaverbeke, J. (2015). 'Looking at biogenic *Lanice conchilega* reefs from a different perspective', *in*: Mees, J. *et al.* (Ed.) (2015). Book of abstracts – VLIZ Young Scientists' Day. Brugge, Belgium, 20 February 2015. VLIZ Special Publication, 71: pp. 49.

Oral presentations

1. De Smet, B., Vincx, M., Vanaverbeke, J. (2012). 'The importance of biogenic reefs in trophic linkages in intertidal sandy beaches'. *First Mares Annual Meeting Science Café.* Ghent, Belgium, 6 February 2012.

2. De Smet, B., Vincx, M., Vanaverbeke, J. (2012). 'The importance of biogenic reefs in trophic linkages in intertidal sandy beaches: current state and future prospective'. *10th Marine Biology Section Symposium (DECEMBSS).* Ghent, Belgium, 30 March 2012.

3. De Smet, B., Vincx, M., Vanaverbeke, J. (2014). 'New insights in the structuring role of *Lanice conchilega* reefs in intertidal food-webs: a focus on epi- and hyperbenthos'. *12th Marine Biology Section Symposium (DUODECEMBSS).* Ghent, Belgium, 21 February 2014.

4. De Smet, B., De Troch, M., Vincx, M., Vanaverbeke, J. (2014). 'Life through isotope-colored glasses: revealing the *Lanice conchilega* food web complexity'. *First Mares conference on Marine Ecosystems Health and Conservation*. Olhão, Portugal, 17-21 November 2014. Book of Abstracts pp. 33.

5. De Smet, B., De Troch, M., Vincx, M., Vanaverbeke, J. (2015). 'Living in an isotopically ordered world: the link between biogenic *Lanice conchilega* reefs and food web structure of sandy beach ecosystems' (Abstract ID:26601). *Aquatic Sciences Meeting (ASLO)*. Granada, Spain, 22-27 February 2015. Program Book, pp. 56.

6. De Smet, B., Fournier, J., De Troch, M., Vincx, M., Vanaverbeke, J. (2015). 'Integrating ecosystem engineering and food webs: testing the effect of biogenic *L. conchilega* reefs on a temperate sandy beach food web'. *VII International Sandy Beach Symposium (ISBS)*. Ilhabela (São Paolo), Brazil, 06-10 July 2015.

Media

1. Knack (28.01.15) – Een rif van schelpkokerwormen op onze kust

This PhD thesis aims at improving our understanding of the structural and functional role of reefs constructed by the ecosystem engineer *Lanice conchilega* in natural soft substrate coastal ecosystems. This was achieved by keeping in mind a holistic approach regarding the formulation of the ecological hypotheses throughout the thesis. At first, the community and ecosystem ecology of *L. conchilega* was studied, followed by characterising trophic interactions taking place within the reef habitats and comprehensively combining ecosystem engineering and food webs. The general discussion combines the results of this thesis and discusses the role of *L. conchilega* reefs regarding the community composition, feedback effects, food web structure and carbon cycling in intertidal areas.

This PhD research has been promoted by Prof. Dr. Magda Vincx and Dr. Jan Vanaverbeke, and was carried out at the Marine Biology Research Group of Ghent University (Belgium). Bart De Smet is trained as a biologist (BSc) and a Marine Scientist (Erasmus Mundus MSc in Marine Biodiversity and Conservation support was provided by the Special Research Fund (BOF) of Ghent University. The thesis has been submitted in partial fulfilment of the requirements for the degree of Doctor in Science (Marine Sciences).